Compass Software for Peggy User Guide

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Compass Software for Peggy User Guide

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Chapter 1:

Getting Started with Compass

Chapter Overview

- Launching the Software
- Software Overview
- Software Menus
- Changing the Compass Main Window Layout
- Software Help
- Checking for and Installing New Versions of Compass
- Viewing Release Notes
- Viewing the Software Log
- Compass Version Information
- Directory and File Information

Launching the Software

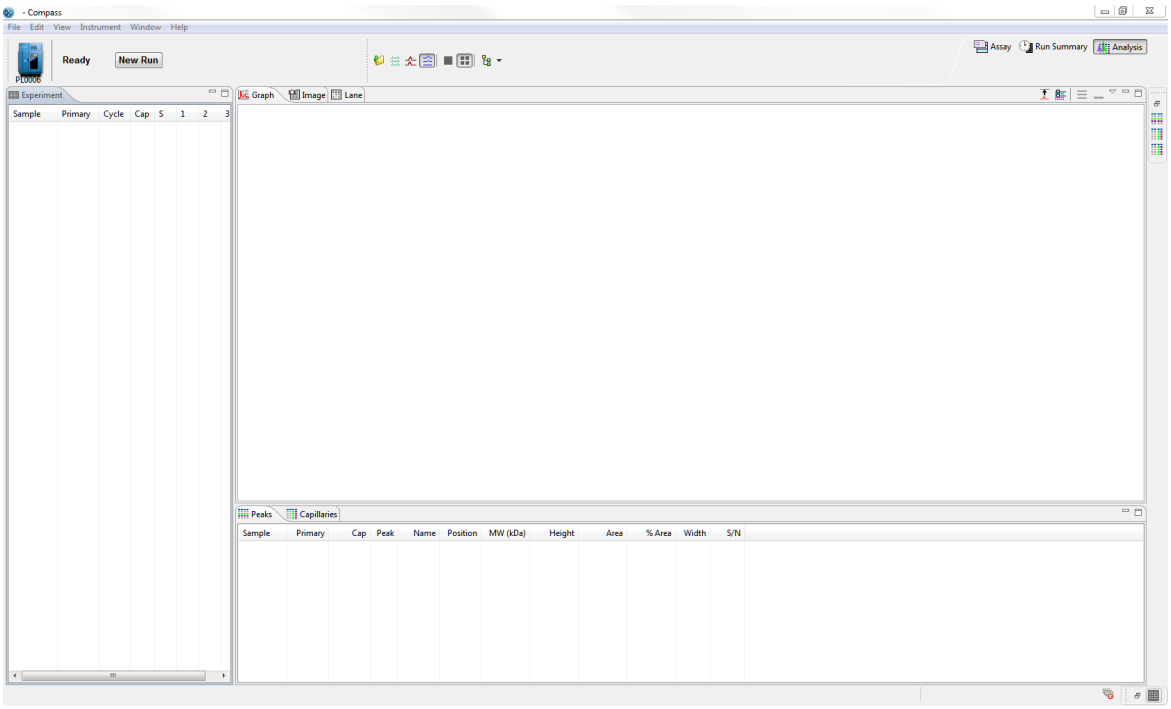
To open Compass, double-click the icon on the computer desktop.

Software Overview

Compass has three main screens:

- **Assay** - For creating and reviewing assays.
- **Run Summary** - For viewing run status.
- **Analysis** - For viewing experimental data.

When Compass opens, the Analysis screen displays by default:

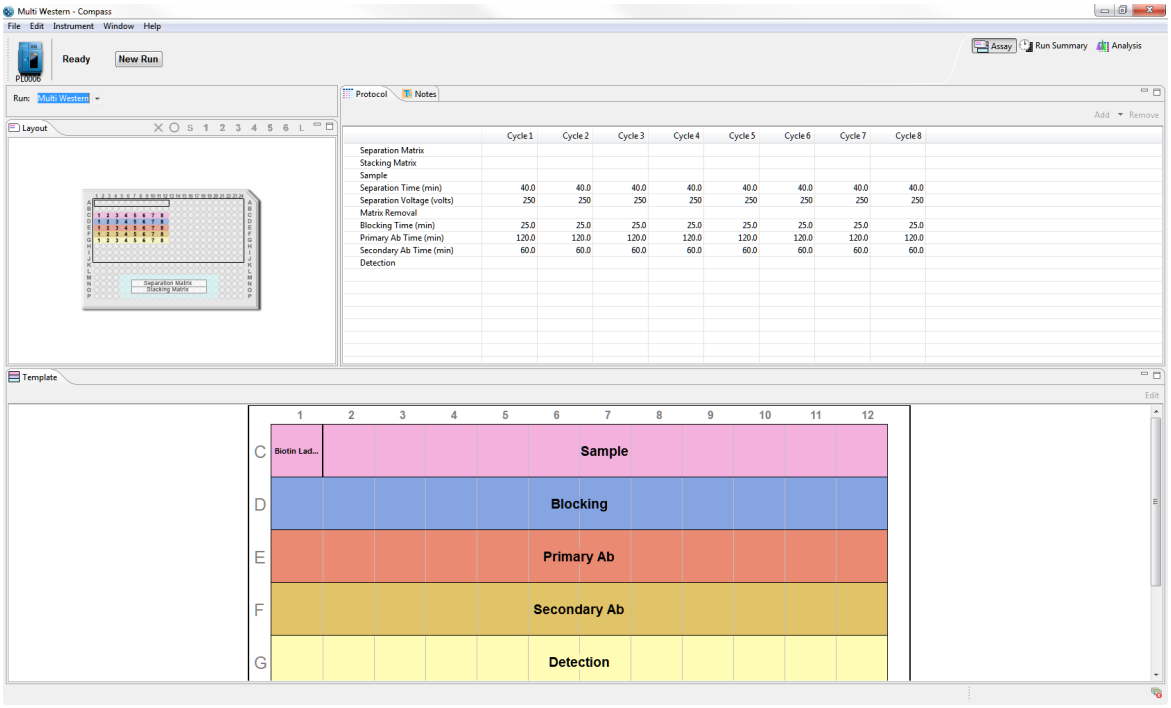


Changing the Screen View

To move between the Assay, Run Summary and Analysis screens, just click the button in the screen tab located in the upper right corner of the main window.

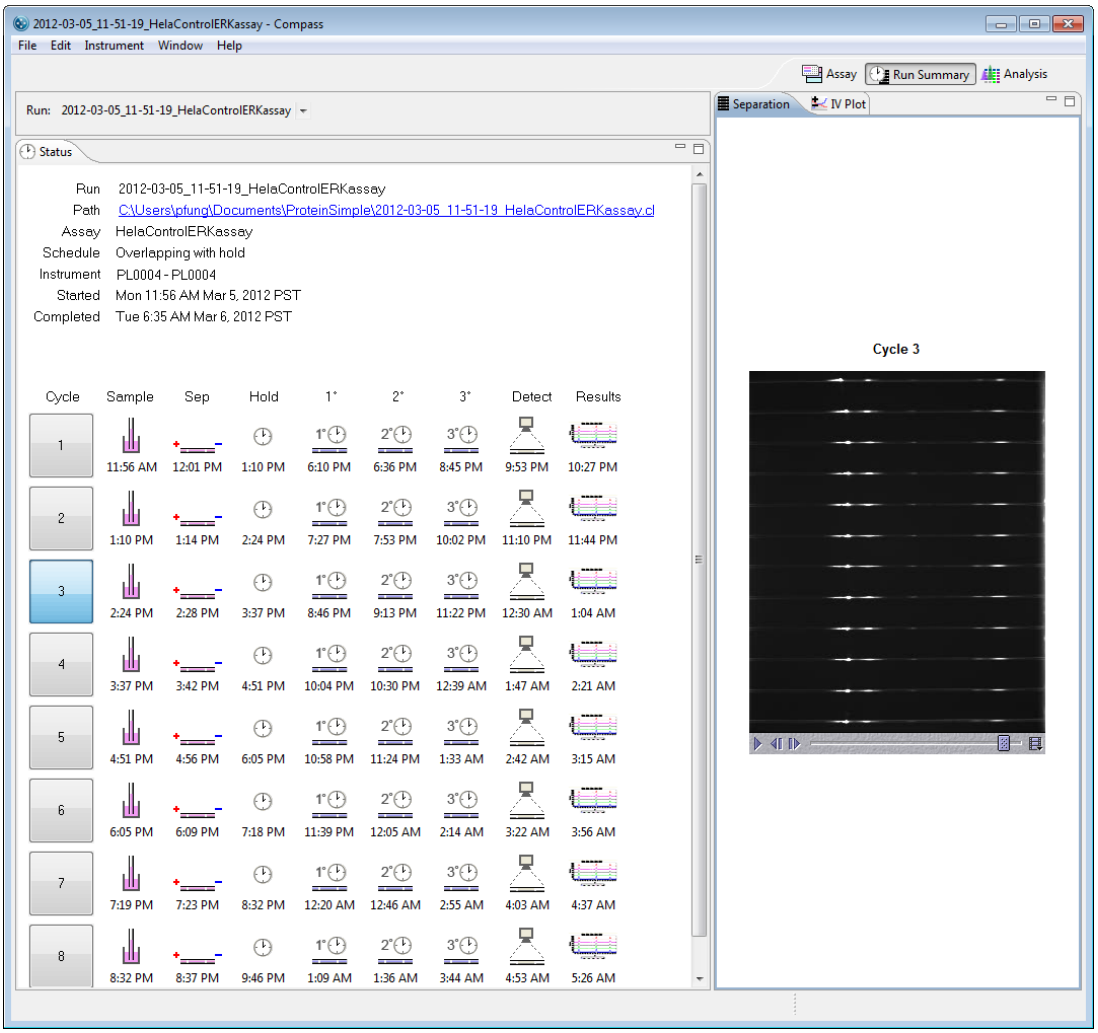
Assay Screen

The Assay screen is used to create, view, and edit assays. Users can assign well locations for assay plate reagents, modify assay protocol steps, enter assay notes and add annotations for individual wells on the assay plate.



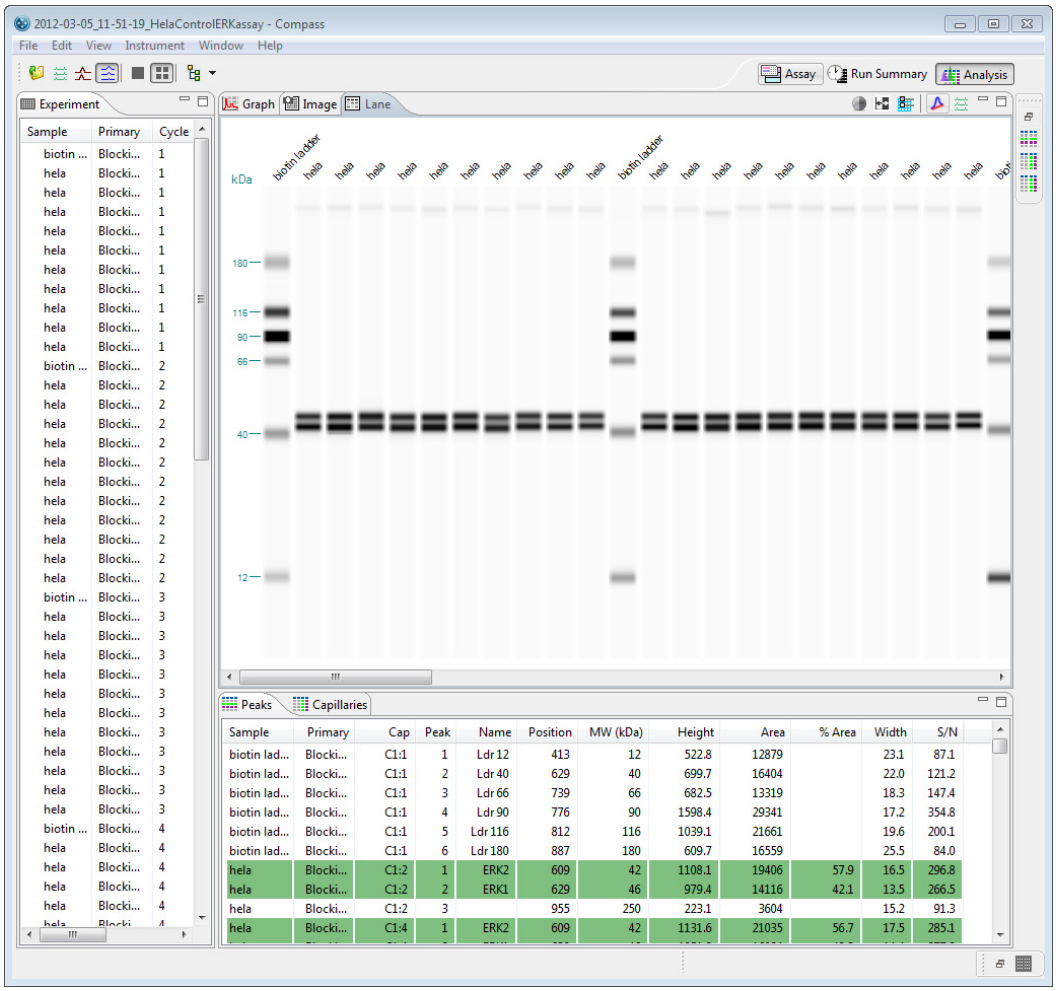
Run Summary Screen

The Run Summary screen is used to monitor status of a run in progress, watch movies of the separation in the capillaries, and view current and voltage plots for each run.


2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit Instrument Window Help
Assay Run Summary Analysis
Run: 2012-03-05_11-51-19_HelaControlERKassay
Separation IV Plot
Status
Run 2012-03-05_11-51-19_HelaControlERKassay
Path C:\Users\pfung\Documents\ProteinSimple\2012-03-05_11-51-19_HelaControlERKassay.cl
Assay HelaControlERKassay
Schedule Overlapping with hold
Instrument PL0004 - PL0004
Started Mon 11:56 AM Mar 5, 2012 PST
Completed Tue 6:35 AM Mar 6, 2012 PST
Cycle 3
Cycle Sample Sep Hold 1° 2° 3° Detect Results
1 11:56 AM 12:01 PM 1:10 PM 6:10 PM 6:36 PM 8:45 PM 9:53 PM 10:27 PM
2 1:10 PM 1:14 PM 2:24 PM 7:27 PM 7:53 PM 10:02 PM 11:10 PM 11:44 PM
3 2:24 PM 2:28 PM 3:37 PM 8:46 PM 9:13 PM 11:22 PM 12:30 AM 1:04 AM
4 3:37 PM 3:42 PM 4:51 PM 10:04 PM 10:30 PM 12:39 AM 1:47 AM 2:21 AM
5 4:51 PM 4:56 PM 6:05 PM 10:58 PM 11:24 PM 1:33 AM 2:42 AM 3:15 AM
6 6:05 PM 6:09 PM 7:18 PM 11:39 PM 12:05 AM 2:14 AM 3:22 AM 3:56 AM
7 7:19 PM 7:23 PM 8:32 PM 12:20 AM 12:46 AM 2:55 AM 4:03 AM 4:37 AM
8 8:32 PM 8:37 PM 9:46 PM 1:09 AM 1:36 AM 3:44 AM 4:53 AM 5:26 AM

Analysis Screen

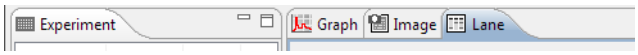
The Analysis screen is used to view run data including electropherograms, capillary images, lane view data and tabulated results. Any post-run analysis is also done in this screen.



Screen Panes

Assay, Run Summary and Analysis screens each contain multiple screen panes that allow users to view the individual components of a run, assay or data file. Each pane has a labeled tab and a unique icon. The panes specific to each screen will be described in the individual screen sections.

The active pane in a screen is blue. To view a pane, click in the pane or on its tab. The example below shows panes in the Analysis screen, and the Lane pane is active:

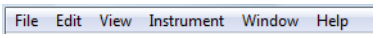


Title Bar

The title bar displays the run file name, and allows the main Compass window to be minimized, maximized or closed.

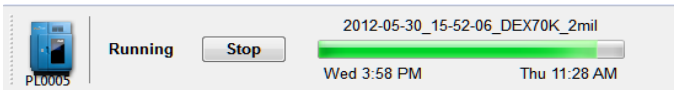
Main Menu

The main menu contains individual menus that provide access to various software, instrument and screen operations. More details on menu commands can be found in "Software Menus" on page 7.



Instrument Status Bar

The instrument status bar is used to start runs and cleaning protocols, relay system status and show run progress. More details on instrument control and status can be found in Chapter 7, *"Controlling Peggy"*.



NOTE: The instrument status bar displays only when Compass software is connected to an instrument. The status bar does not display on computer workstations used just for data analysis.

Screen Tab

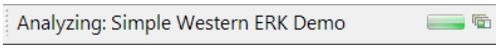
The screen tab lets you move between Assay, Run Summary or Analysis screens and is located in the upper right corner of the main window. Just click a button to view a screen.

View Bar

The view bar is only displayed in the Analysis screen as part of the main menu bar and allows users to switch between displaying sample chemiluminescent data, fluorescent standards or capillary registration information, data for a single capillary or all capillaries in the run, or grouped capillary data. View bar options are detailed in “Switching Between Sample, Standards and Registration Data Views” on page 146 for size assays or page 278 for charge assays, and “Using Groups” on page 175 for size assays or page 304 for charge assays.

Compass Status Bar

The status bar is located in the lower right corner of the main window. It displays active software processes and their progress.

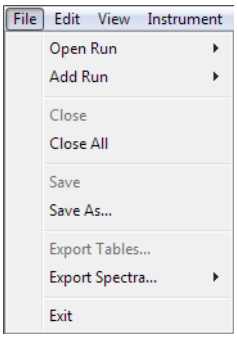


Software Menus

A brief description of the software menus in the main menu are described in this section. Not all menus are available in every screen, and menu commands change depending on what screen is active. The menus and commands available for each screen will be detailed in the individual screen sections.

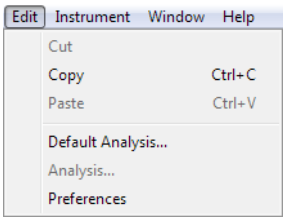
File Menu

The File menu contains basic file commands.



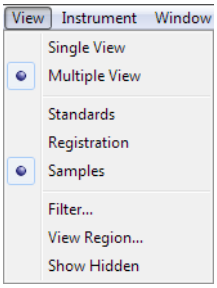
Edit Menu

The Edit menu contains basic editing commands, analysis and preferences options. Preferences is described in Chapter 10, *"Setting Custom User Preferences"*.



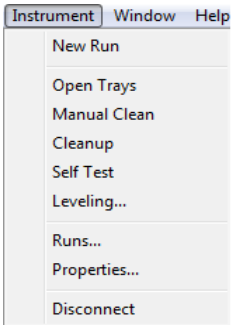
View Menu

The View menu is only available in the Analysis screen, and allows users to change how data is displayed.



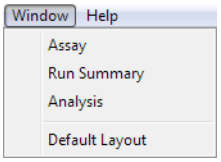
Instrument Menu

The Instrument menu is only available when Compass is connected to Peggy directly. Instrument control options are explained in Chapter 7, *"Controlling Peggy"*.



Window Menu

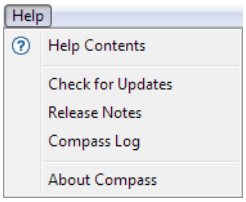
The Window menu allows users to switch between Assay, Run Summary or Analysis screens, and restore screens to the Compass default layout.



- **Assay** - Displays the Assay screen which is used to create, view, and edit assays.
- **Run Summary** - Displays the Run Summary screen which is used to monitor the status of a run in progress.
- **Analysis** - Displays the Analysis screen which is used to view sample electropherograms, lane data and results.
- **Default Layout** - Restores the individual panes in the current screen back to their default size and location.

Help Menu

The Help menu provides access to Help, software updates, release notes and other software information.



- **Help Contents** - Displays the Compass Help file.
- **Check for Updates** - Automatically checks to see if a new version of Compass is available.
- **Release Notes** - Displays Compass release notes for the current and prior versions.
- **Compass Log** - Displays the Compass software log file.
- **About Compass** - Displays the Compass software version and build information.

Changing the Compass Main Window Layout

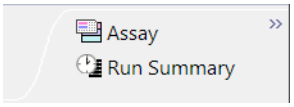
The Compass main window can easily be resized, as can the individual panes in each screen. Screen panes can also be moved outside of the main window.

Resizing the Main Compass Window

To resize the main window, roll the mouse over a corner or border until the sizing arrow appears. Then just click and drag to resize.

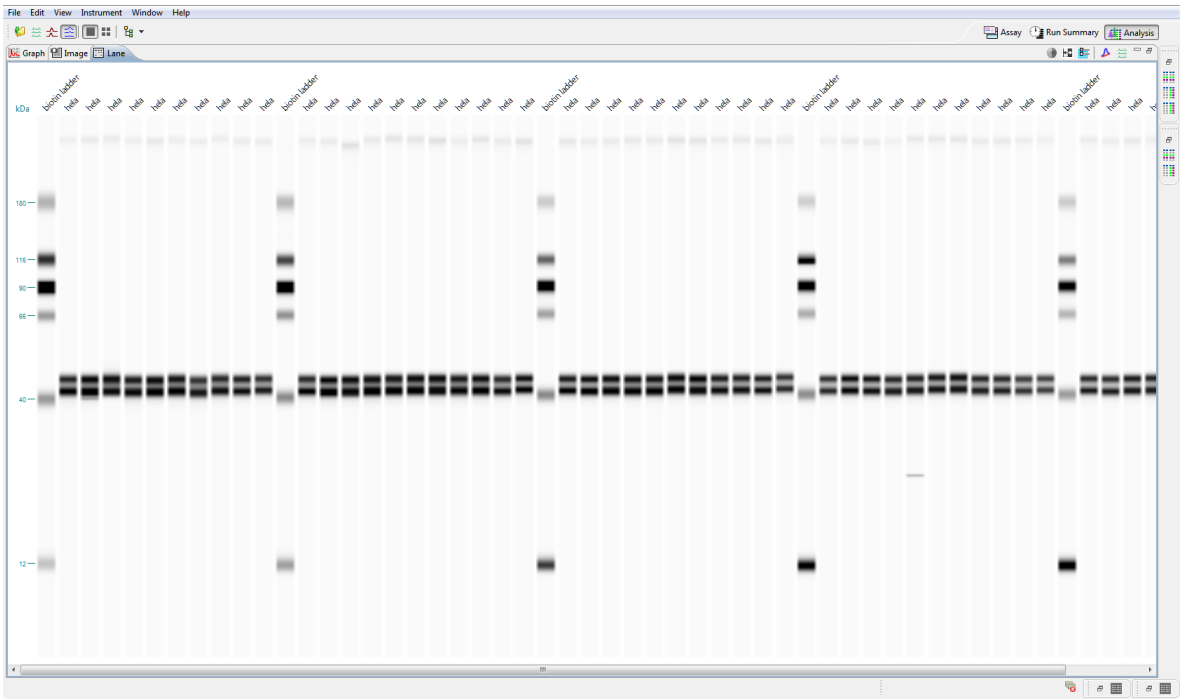
Resizing the Screen Tab

The screen tab can be sized to show all or just some of the screen buttons. To resize, roll the mouse over the left edge of the tab until the sizing arrow appears, then click and drag to resize. If a screen button is hidden, a double arrow will display in the tab. Click to display and select the hidden screen.



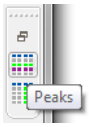
Resizing Screen Panes

- **To resize a pane** - Roll the mouse over the pane border until the sizing arrow appears. Then just click and drag to resize.
- **To maximize a pane** - Click the maximize button in the upper right corner or double-click the tab. The other panes in the screen will automatically minimize to pane bars in the task area along the window border.

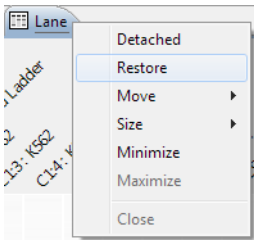


- **To restore all minimized panes** - Click **Restore** on the minimized pane bar.

- **To restore only one minimized pane** - Click the pane icon on the minimized pane bar.



- **To restore a maximized pane to its original size** - Double-click the tab or right click the tab and click **Restore**.



- **To restore all panes to their original sizes -** Select **Window** in the main menu and click **Default Layout**.

Changing the Location of Screen Panes

Panes can be moved to different locations within a screen.

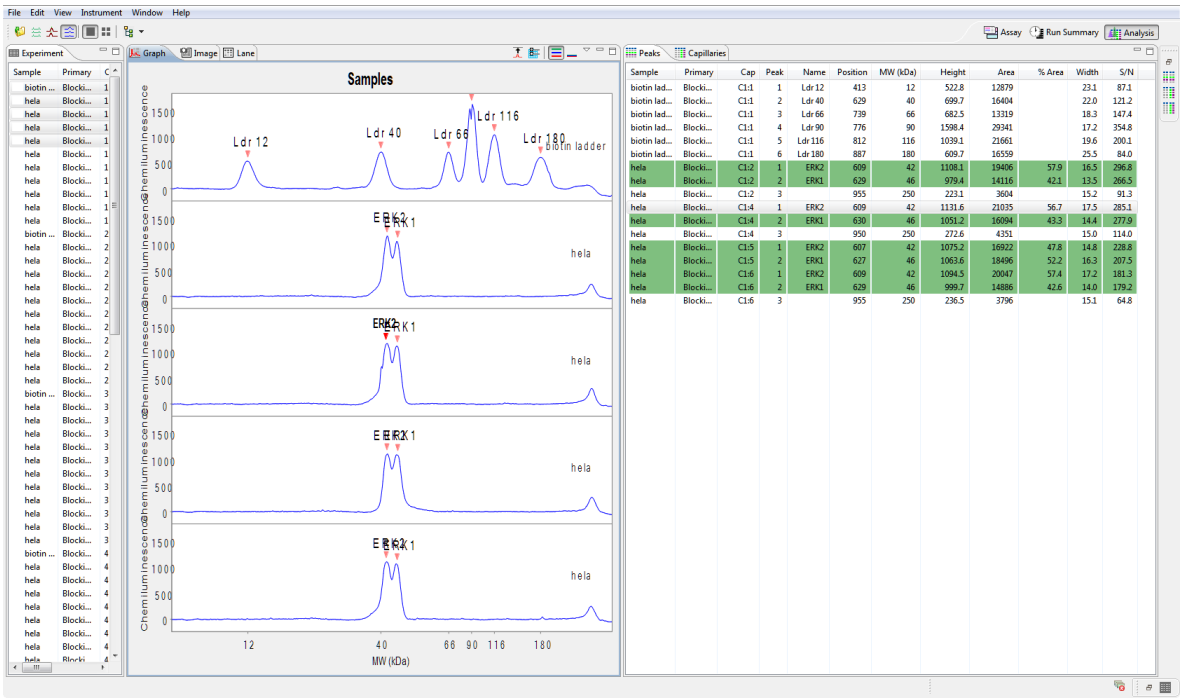
- **To move a pane** - Click on its tab and drag it to the new location. As the pane is moved, area guides will display to assist users in choosing a drop location.

Area guides with a black arrow let you know that if the pane is dropped at that location, it will be resized and relocated as an individual pane in that area of the screen.

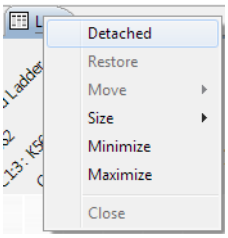
Area guides with a folder let you know that if the pane is dropped at that location, it will be added as a new tab in an area with one or more pane tabs.

Area guides with a window let you know that if the pane is dropped at that location, it will become a separate window outside the Compass main window.

The following figure shows the Analysis screen after moving the Graph pane.



- **To detach a pane from the main window -** Click on its tab and drag it outside the main Compass window or right click the tab and click **Detached**.



- **To move a detached pane back inside the main window -** Right click the tab and deselect **Detached**.
- **To restore all panes to their original locations -** Select **Window** in the main menu and click **Default Layout**.

Restoring the Main Window to the Default Layout

To restore screen pane sizes and locations to the original Compass layout, select **Window** from the main menu and click **Default Layout**.

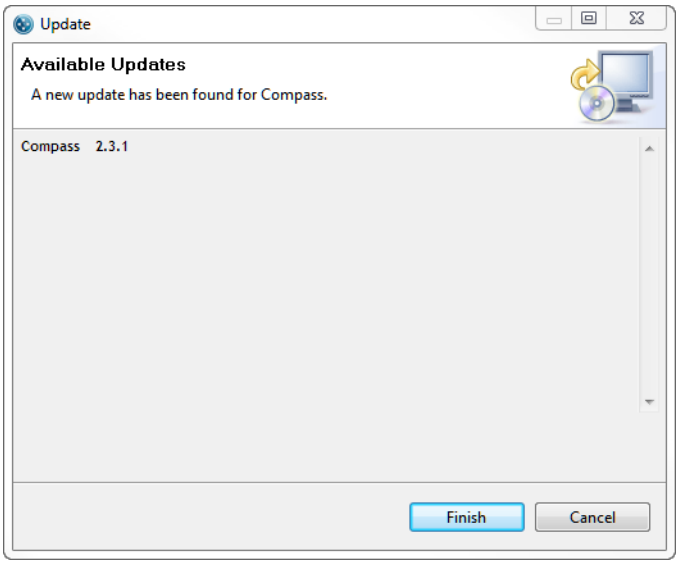
Software Help

Select **Help** and click **Help Contents** to view the Compass Help file.

Checking for and Installing New Versions of Compass

Compass can automatically check to see if a newer version of software is available. To do this:

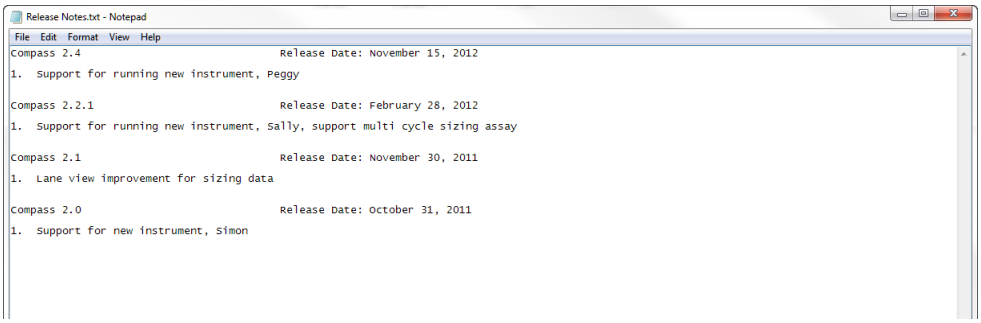
1. Make sure the computer being used has an active internet connection.
2. Select **Help** and click **Check for Updates**. If an update is found, the following screen will display:



3. Click **Finish** to start the download and install the update.
4. Follow the on-screen instructions to complete the software installation.
5. Reboot the computer before using the new version of Compass.

Viewing Release Notes

Select **Help** and click **Release Notes** to view feature updates and bug fixes for new and past versions of Compass. ProteinSimple recommends users review these notes whenever a software update is installed.

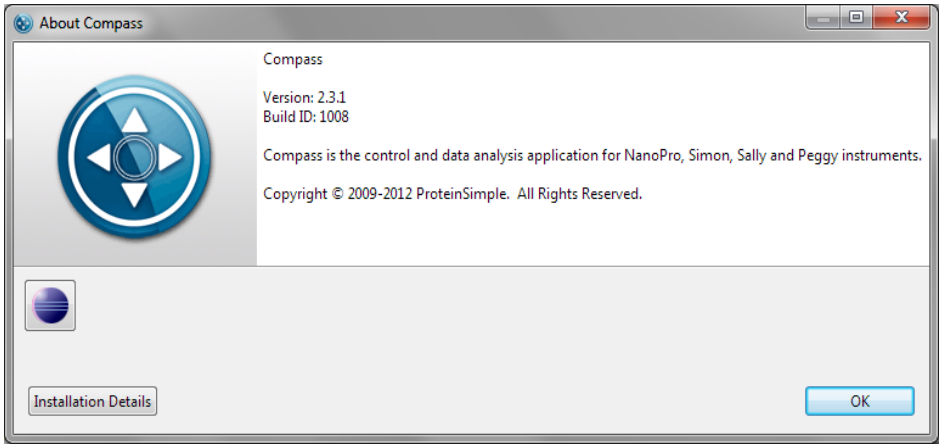

Release Notes.txt - Notepad
File Edit Format View Help
Compass 2.4 Release Date: November 15, 2012
1. Support for running new instrument, Peggy
Compass 2.2.1 Release Date: February 28, 2012
1. Support for running new instrument, Sally, support multi cycle sizing assay
Compass 2.1 Release Date: November 30, 2011
1. Lane view improvement for sizing data
Compass 2.0 Release Date: October 31, 2011
1. Support for new instrument, Simon

Viewing the Software Log

Select **Help** and click **Compass Log** to view the software log file.

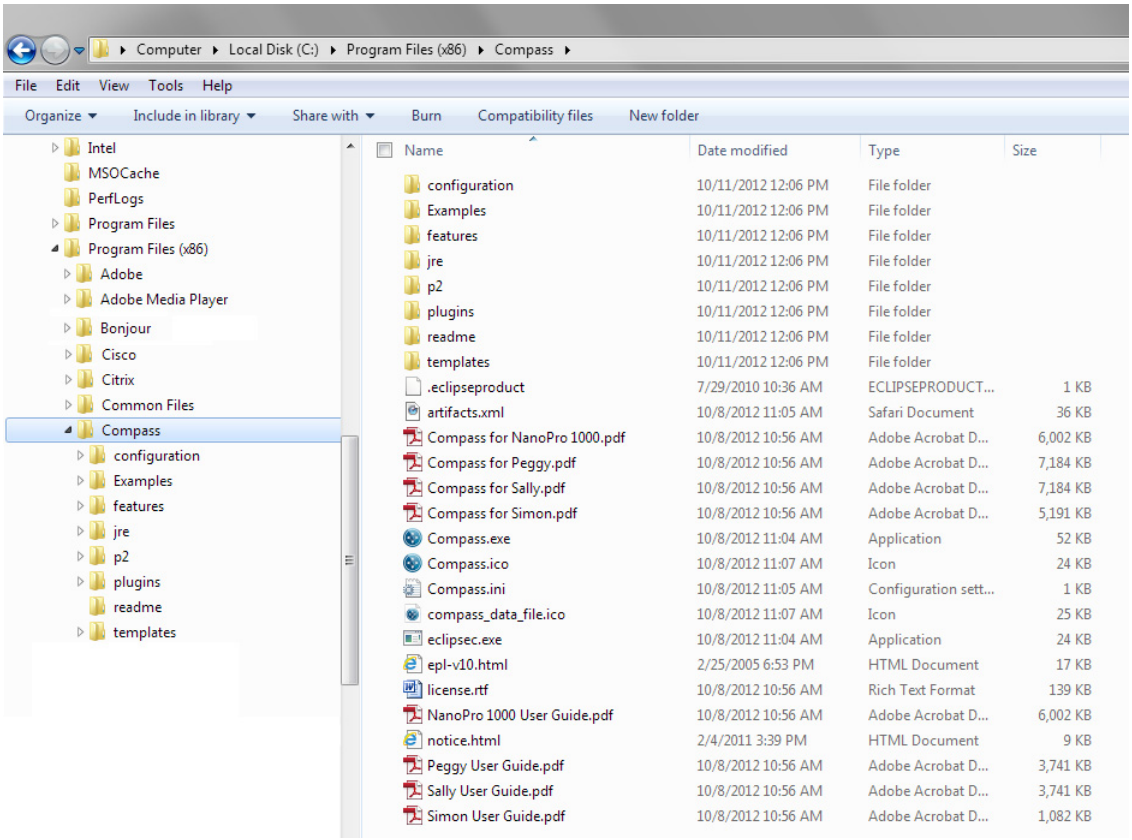
Compass Version Information

Select **Help** and click **About Compass** to view the software version and build number information.

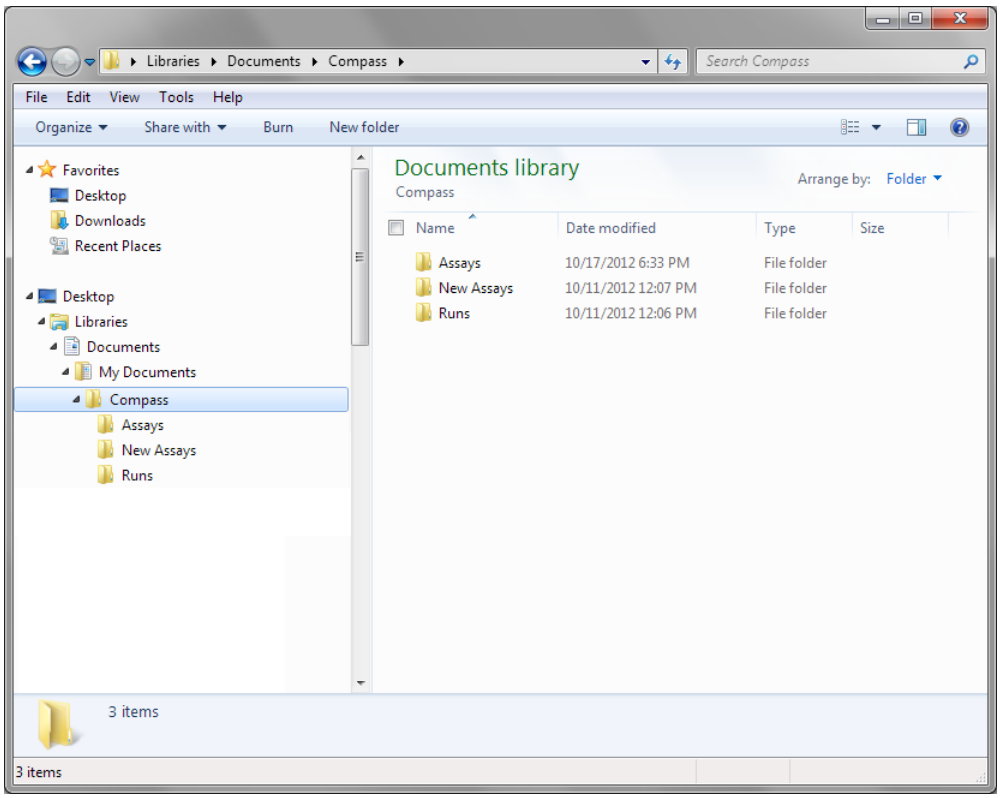

About Compass
Compass
Version: 2.3.1
Build ID: 1008
Compass is the control and data analysis application for NanoPro, Simon, Sally and Peggy instruments.
Copyright © 2009-2012 ProteinSimple. All Rights Reserved.
Installation Details
OK

Directory and File Information

The main Compass directory is located in the Program Files folder, and also contains a PDF file of the Peggy User Guide.


Computer ▸ Local Disk (C:) ▸ Program Files (x86) ▸ Compass ▸
File Edit View Tools Help
Organize Include in library Share with Burn Compatibility files New folder
Intel
MSOCache
PerfLogs
Program Files
Program Files (x86)
Adobe
Adobe Media Player
Bonjour
Cisco
Citrix
Common Files
Compass
configuration
Examples
features
jre
p2
plugins
readme
templates
Name Date modified Type Size
configuration 10/11/2012 12:06 PM File folder
Examples 10/11/2012 12:06 PM File folder
features 10/11/2012 12:06 PM File folder
jre 10/11/2012 12:06 PM File folder
p2 10/11/2012 12:06 PM File folder
plugins 10/11/2012 12:06 PM File folder
readme 10/11/2012 12:06 PM File folder
templates 10/11/2012 12:06 PM File folder
.eclipseproduct 7/29/2010 10:36 AM ECLIPSEPRODUCT... 1 KB
artifacts.xml 10/8/2012 11:05 AM Safari Document 36 KB
Compass for NanoPro 1000.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 6,002 KB
Compass for Peggy.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 7,184 KB
Compass for Sally.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 7,184 KB
Compass for Simon.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 5,191 KB
Compass.exe 10/8/2012 11:04 AM Application 52 KB
Compass.ico 10/8/2012 11:07 AM Icon 24 KB
Compass.ini 10/8/2012 11:05 AM Configuration sett... 1 KB
compass_data_file.ico 10/8/2012 11:07 AM Icon 25 KB
eclipsec.exe 10/8/2012 11:04 AM Application 24 KB
epl-v10.html 2/25/2005 6:53 PM HTML Document 17 KB
license.rtf 10/8/2012 10:56 AM Rich Text Format 139 KB
NanoPro 1000 User Guide.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 6,002 KB
notice.html 2/4/2011 3:39 PM HTML Document 9 KB
Peggy User Guide.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 3,741 KB
Sally User Guide.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 3,741 KB
Simon User Guide.pdf 10/8/2012 10:56 AM Adobe Acrobat D... 1,082 KB

Compass assay and run files are located in the Documents folder in the User directory:



- **Assays Folder** - Contains all saved assay files.
- **New Assays Folder** - Contains assay template files.
- **Runs Folder** - Contains all run files. Run data is automatically written to this folder.

NOTE: When a Compass software update is performed, the template assays in the New Assays folder are overwritten. If you have customized these assays, ProteinSimple recommends saving them in a unique subfolder prior to updating the software, then transferring them back to the New Assays folder after the update to avoid losing your assay customizations.

File Types

The following file types are used by Compass:

- **Assay Files** - Use an *.assay file extension.
- **Run Files** - Use a *.cbz file extension. The default file format for run files is Date_Time_AssayName. An example run file name would be 2012-09-28_18-50-53_Simple Western.cbz.
- **Protocol Files** - Exported protocol files use a *.protocol file extension.
- **Template Files -** Exported template files use a *.template file extension.
- **Analysis Settings Files** - Exported analysis settings files use a *.settings file extension.

Chapter 2:

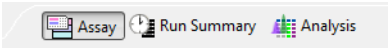
Size Assays

Chapter Overview

- Assay Screen Overview
- Reagent Color Coding
- Opening an Assay
- Creating a New Assay
- Making Changes to an Existing Assay
- Switching Between Open Assays
- Creating a Template Assay
- Viewing and Changing the Detection Exposures
- Copying Protocols and Templates
- Printing Protocols and Templates
- Importing and Exporting Protocols and Templates

Assay Screen Overview

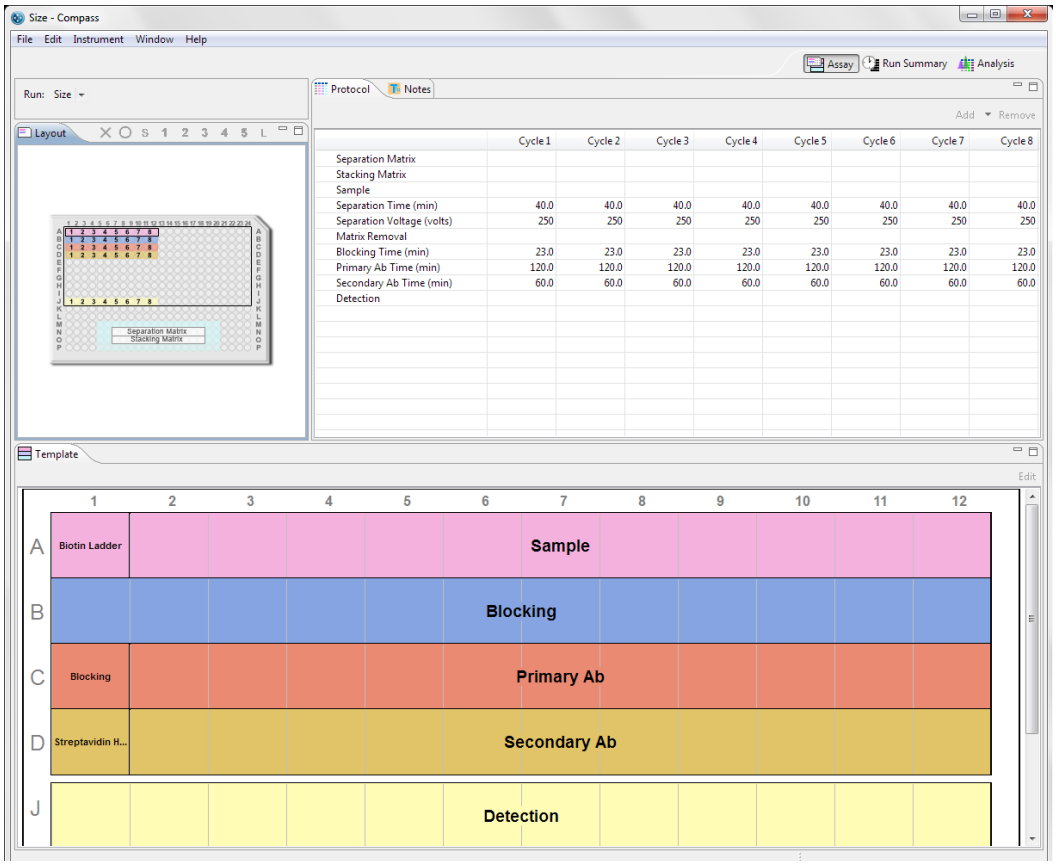
The Assay screen is used to create, view, and edit assays. To access this screen, click **Assay** in the screen tab:



Assay Screen Panes

The Assay screen has four panes:

- **Layout** - Displays a map of the assay plate and shows where assay reagents will be located.
- **Protocol** - Lists individual assay protocol steps and parameters that Peggy will execute for each of the 12 capillaries simultaneously.
- **Notes** - Lets you enter specific assay information that is saved with the assay and can be used for future reference.
- **Template** - Enter annotations for the individual well and row reagents in the assay plate.


Size - Compass
File Edit Instrument Window Help
Assay Run Summary Analysis
Run: Size
Layout
Protocol Notes
Add Remove
Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8
Separation Matrix
Stacking Matrix
Sample
Separation Time (min) 40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Separation Voltage (volts) 250 250 250 250 250 250 250 250
Matrix Removal
Blocking Time (min) 23.0 23.0 23.0 23.0 23.0 23.0 23.0 23.0
Primary Ab Time (min) 120.0 120.0 120.0 120.0 120.0 120.0 120.0 120.0
Secondary Ab Time (min) 60.0 60.0 60.0 60.0 60.0 60.0 60.0 60.0
Detection
Template
Edit
1 2 3 4 5 6 7 8 9 10 11 12
A Biotin Ladder Sample
B Blocking
C Blocking Primary Ab
D Streptavidin H... Secondary Ab
J Detection

Software Menus Active in the Assay Screen

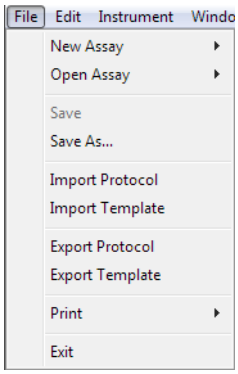
The following software menus are available:

- File
- Edit
- Instrument (when Compass is connected to Peggy)
- Window
- Help

The File and Edit menu options specific to the Assay screen are described next.

File Menu

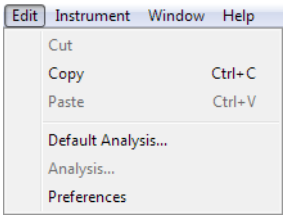
The following File menu options are active:



- **New Assay** - Creates a new assay from a starter template.
- **Open Assay** - Opens an existing assay.
- **Save** - Saves the open assay.
- **Save As** - Saves the open assay under a different file name.
- **Import Protocol** - Imports a saved protocol file into an assay.
- **Import Template** - Imports a saved template file into an assay.
- **Export Protocol** - Exports the current protocol file for future use.
- **Export Template** - Exports the current template file for future use.
- **Print** - Prints the information in the Protocol or Template panes.
- **Exit** - Closes Compass.

Edit Menu

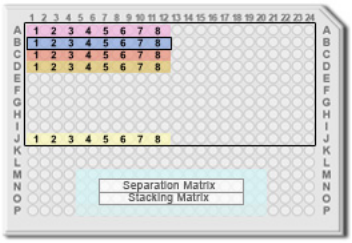
The following Edit menu options are active:



- **Copy** - Copies the information in the Protocol or Template panes into other documents.
- **Default Analysis** - Displays the default settings that will be used to analyze the run data generated with an assay.
- **Analysis** - Not active in this screen.
- **Preferences** - Set and save custom preferences for data export, plot colors in the graph and Peggy's Twitter settings. See Chapter 10, *"Setting Custom User Preferences"* for more information.

Reagent Color Coding

The Assay screen uses color coding to identify various assay reagents in all panes. The Layout pane is shown in the following example:

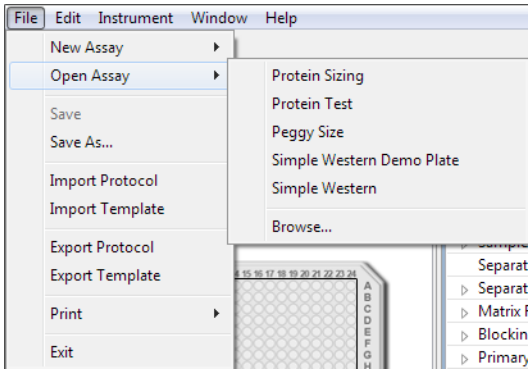


- **Pink** - Samples and Ladder
- **Dark Blue** - Blocking reagent (Antibody Diluent)
- **Orange** - Primary antibody
- **Gold** - Secondary HRP conjugate
- **Yellow** - Luminol/Peroxide mix
- **No color coding** - Separation Matrix (clearly designated)
- **No color coding** - Stacking Matrix (clearly designated)
- **Light Blue** - Water dispensed around Separation and Stacking Matrices

Opening an Assay

To open an existing assay:

1. Select **File** in the main menu and click **Open Assay**.



2. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

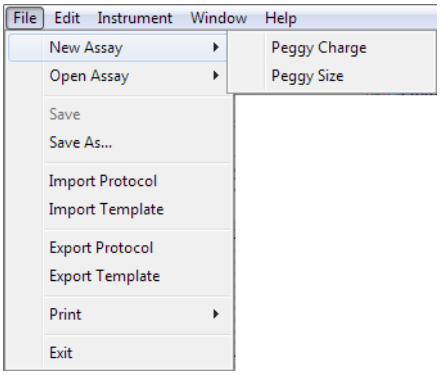
Creating a New Assay

To create a new assay, ProteinSimple recommends using the Peggy template assay and modifying from there as needed.

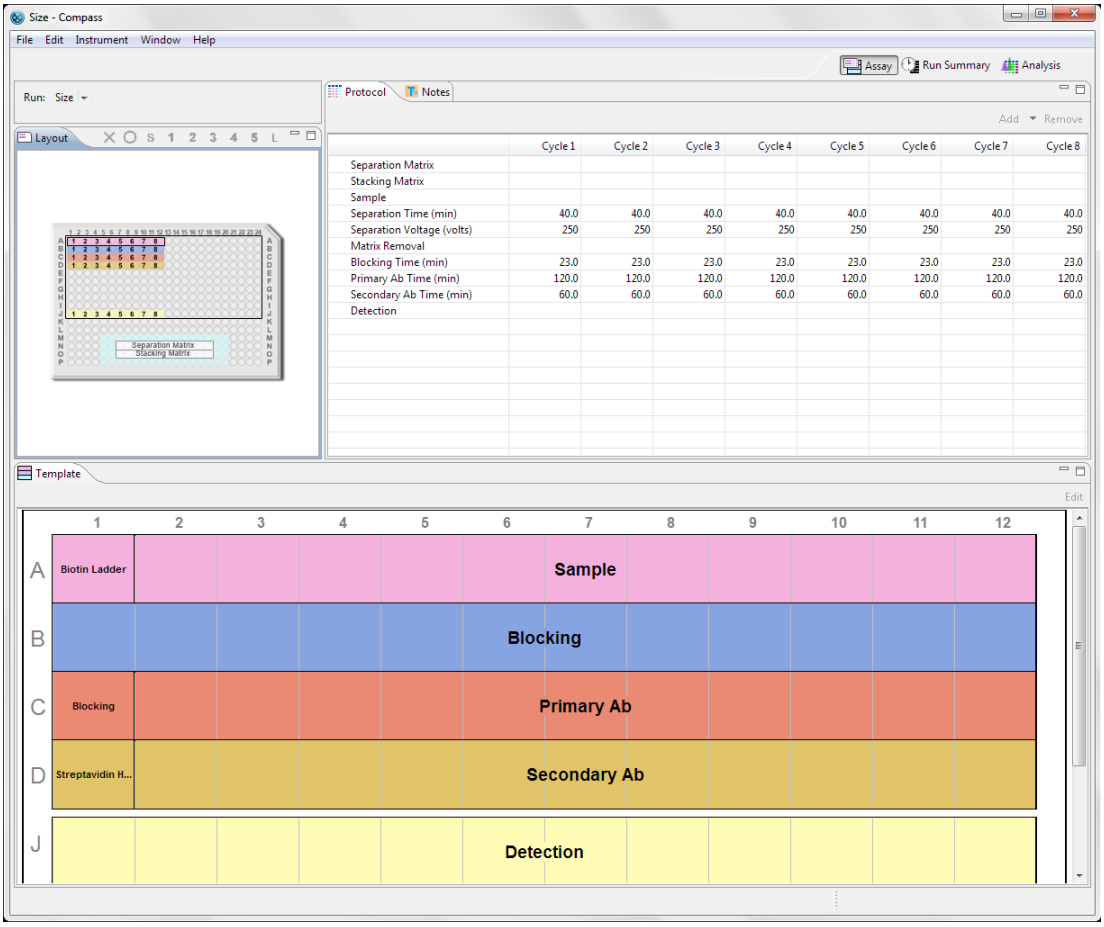
Step 1 - Open a Template Assay

1. Select **File** in the main menu and click **New Assay**:

T



2. A list of template assays that can be used as a starting point for new assays will display. Click **Peggy Size**. The Peggy template assay and default settings will display in the Assay screen:

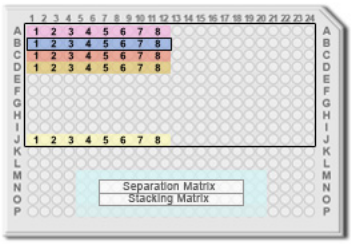

Size - Compass
File Edit Instrument Window Help
Assay
Run Summary
Analysis
Run: Size
Protocol
Notes
Layout
Add
Remove
Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8
Separation Matrix
Stacking Matrix
Sample
Separation Time (min) 40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Separation Voltage (volts) 250 250 250 250 250 250 250 250
Matrix Removal
Blocking Time (min) 23.0 23.0 23.0 23.0 23.0 23.0 23.0 23.0
Primary Ab Time (min) 120.0 120.0 120.0 120.0 120.0 120.0 120.0 120.0
Secondary Ab Time (min) 60.0 60.0 60.0 60.0 60.0 60.0 60.0 60.0
Detection
Template
Edit
1 2 3 4 5 6 7 8 9 10 11 12
A Biotin Ladder Sample
B Blocking
C Blocking Primary Ab
D Streptavidin H... Secondary Ab
J Detection

Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. This pane displays the default row locations of where each reagent should be placed on the assay plate using the Plate Guide provided with Peggy.

NOTE: Up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.

- **Row A** - Sample and Biotinylated Ladder



- **Row B** - Blocking (Antibody Diluent)
- **Row C**- Antibody Diluent (C1) and Primary antibody (C2-C12)
- **Row D** - Streptavidin-HRP (D1) and Secondary HRP conjugate (D2-D12)
- **Row J** - Luminol-S/Peroxide mix
- **Row M** - Water (M5-M20)
- **Row N** - Water (N5-N6 and N19-N20) and Separation Matrix (N7-N18)
- **Row O** - Water (O5-O6 and O19-O20) and Stacking Matrix (O7-O18)
- **Row P** - Water (P5-P20)

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

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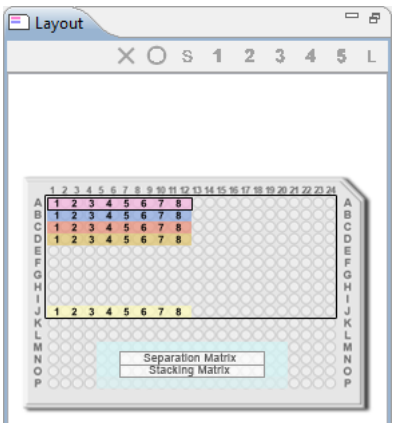
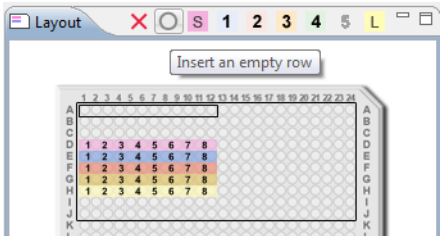
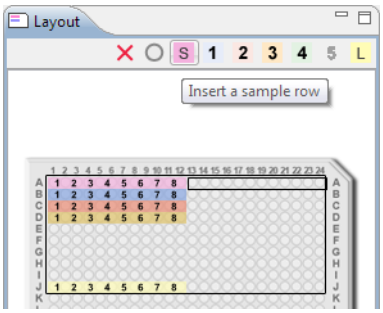
2. If needed, well assignments can be modified as described below. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
   - **To move a reagent row to another location** - Click the row in the **Layout** pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.



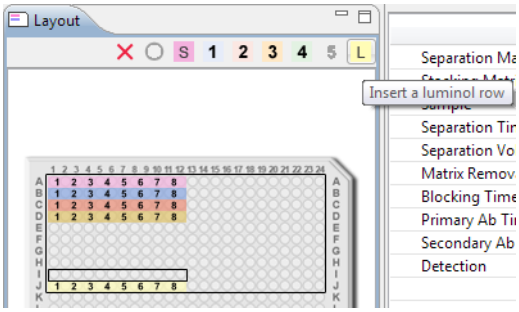
- **To insert a new row** - Click the row below where the new one should be inserted, then click **Insert an empty row** (circle icon) in the **Layout** pane toolbar. A new row will be inserted above the selected row.



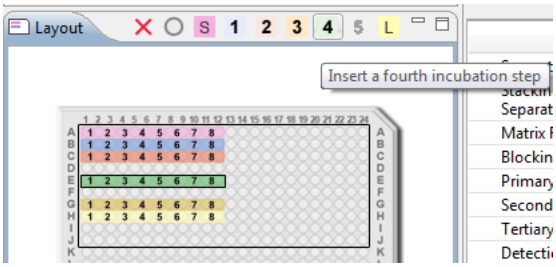
- **To insert a sample row -** Click an empty row or the row below where the new sample row should be inserted, then click **Insert a sample row** (S icon) in the **Layout** pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.



- **To insert a luminol row -** Click an empty row or the row below where the new luminol row should be inserted, then click **Insert a luminol row** (L icon) in the **Layout** pane toolbar. A new luminol row will be added in the empty row or inserted above the selected row.



- **To insert a fourth or fifth incubation reagent** - Click an empty row or the row below where the new incubation reagent should be inserted, then click **Insert a 4th incubation step** (4 icon) in the **Layout** pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.

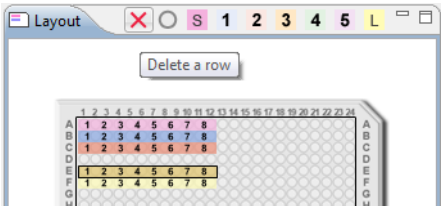


A fifth incubation reagent can now be added by repeating the above and clicking **Insert a 5th incubation step** (5 icon) instead.

NOTES:
When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

- **To delete a row** - Click the row to be deleted, then click **Delete** (red x icon) in the toolbar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.



NOTES:
Samples, antibodies and blocking buffer can be dispensed in Rows A-J and in columns 1-12 or 13-24.

ProteinSimple recommends keeping two rows empty between the substrate and the closest dispensed HRP conjugate to avoid any interaction with the substrate.

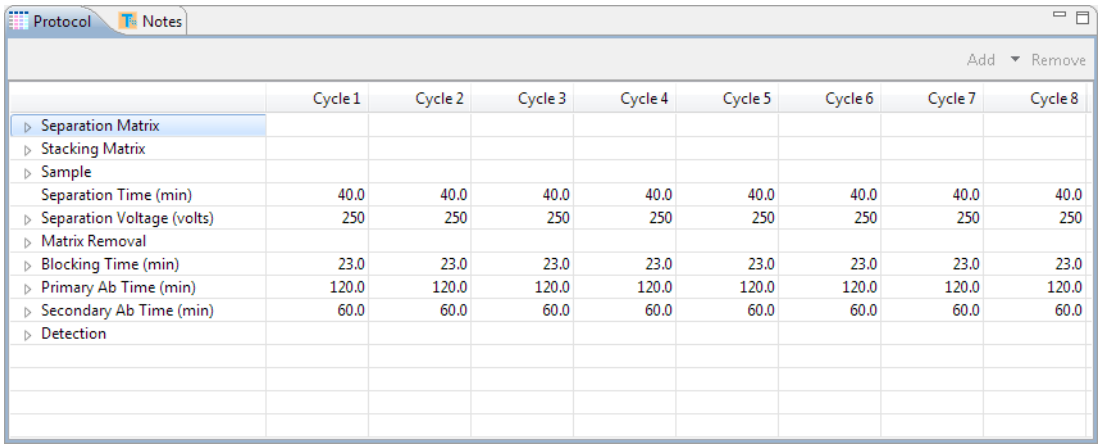
Rows K and L are purposely left empty, do not pipet reagents into these rows of wells.

Separation and Stacking matrices must be dispensed in the designated wells.

Water is dispensed to reduce the potential of evaporation of the matrices.

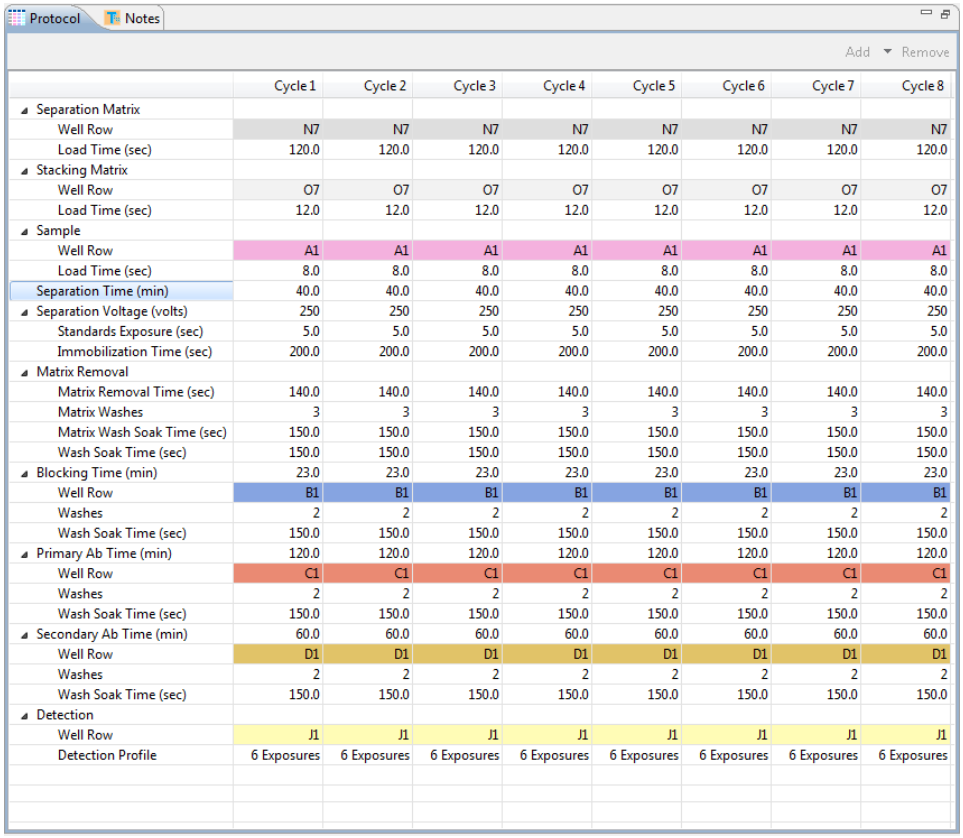
Step 3 - Modifying the Assay Protocol (Optional)

1. Click on the Protocol tab. This pane displays the individual steps of the assay protocol and allows you to change parameters as needed. When creating a new assay, a default protocol will display which automatically assigns all reagent locations for Cycles 1-8:



	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Blocking Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Detection								

Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. ProteinSimple recommends using the default protocol settings for Peggy assays. An expanded list of the default protocol step parameters is shown:

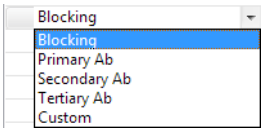


	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7	N7	N7	N7	N7	N7	N7	N7
Load Time (sec)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Stacking Matrix								
Well Row	O7	O7	O7	O7	O7	O7	O7	O7
Load Time (sec)	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Sample								
Well Row	A1	A1	A1	A1	A1	A1	A1	A1
Load Time (sec)	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Standards Exposure (sec)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Immobilization Time (sec)	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Matrix Removal								
Matrix Removal Time (sec)	140.0	140.0	140.0	140.0	140.0	140.0	140.0	140.0
Matrix Washes	3	3	3	3	3	3	3	3
Matrix Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Blocking Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								
Well Row	J1	J1	J1	J1	J1	J1	J1	J1
Detection Profile	6 Exposures	6 Exposures	6 Exposures	6 Exposures	6 Exposures	6 Exposures	6 Exposures	6 Exposures

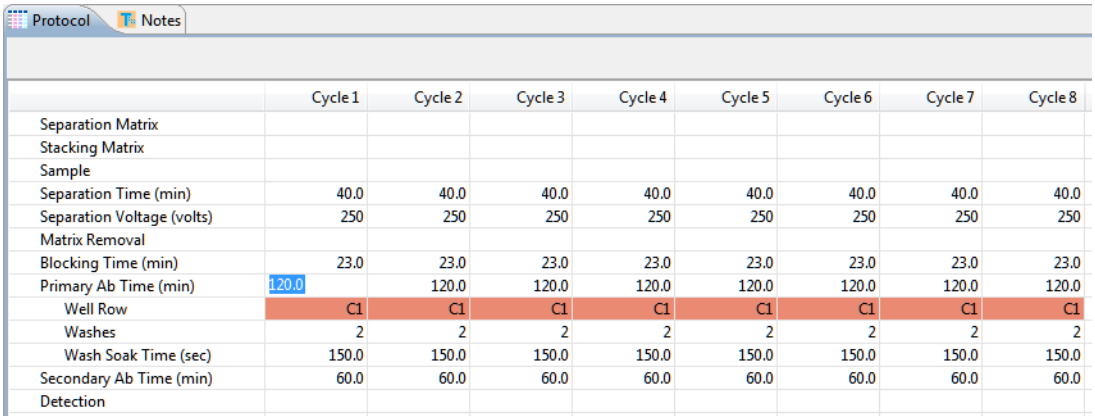
2. Five incubation steps are allowed per protocol. Users can select the type of incubation for each step. The available incubation types and their default Simple Western use is as follows:

   - **First incubation** - Blocking
   - **Second incubation** - Primary antibody
   - **Third incubation** - Secondary antibody
   - **Fourth incubation** - User defined (tertiary antibody)
   - **Fifth incubation** - User defined (custom)

   To change the type, click the incubation step name and select an option from the drop down list.



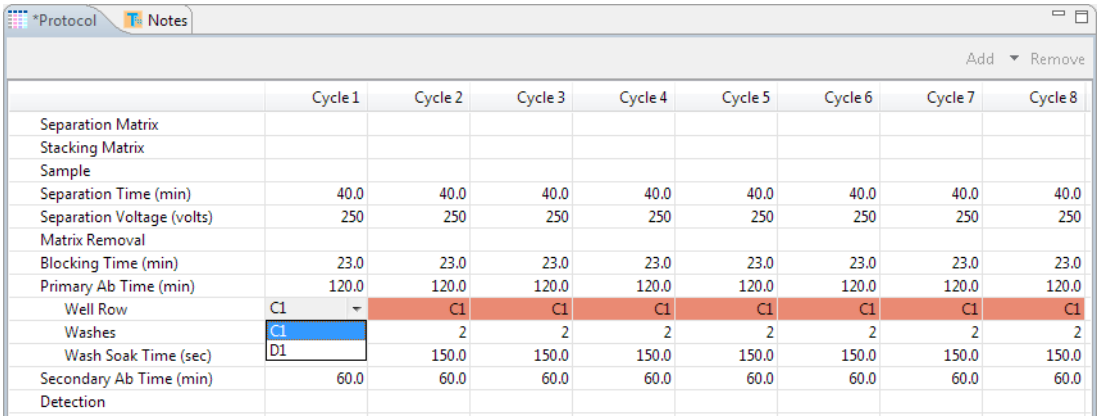
3. If needed, change the primary incubation time. To do this, click the cell in the value column next to Primary Ab Time (min) and enter a new value in minutes:



Protocol | Notes

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Blocking Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Detection								

4. If needed, change the primary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:



*Protocol | Notes

Add Remove

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Blocking Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	C1	2	2	2	2	2	2	2
Wash Soak Time (sec)	D1	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Detection								

NOTE: Only rows designated as primary antibody in the Layout tab can be selected in the Well Row drop-down menu.

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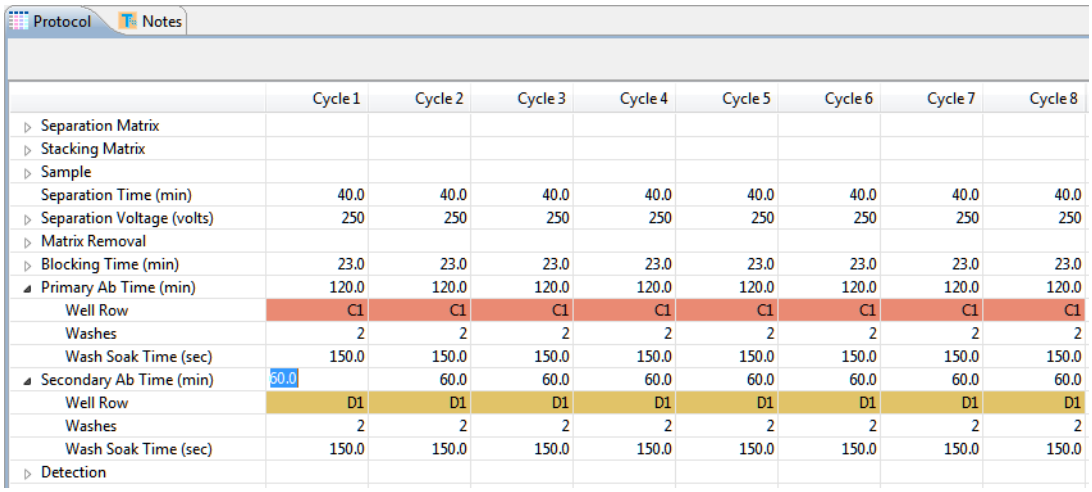
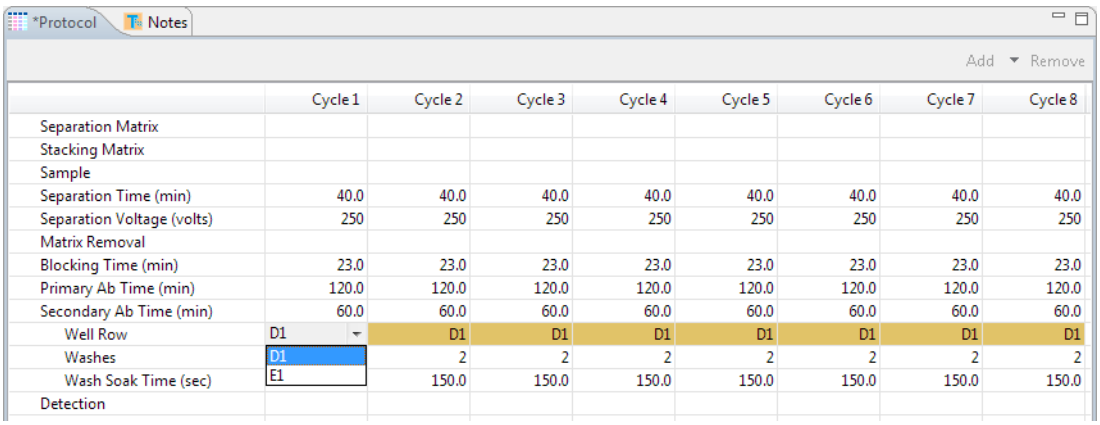
5. If needed, change the secondary incubation time. To do this, click the cell in the value column next to Secondary Ab Time (min) and enter a new value in minutes:



Protocol | Notes

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Blocking Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								

6. If needed, change the secondary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:



*Protocol | Notes

Add | Remove

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Blocking Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	D1	2	2	2	2	2	2	2
Wash Soak Time (sec)	E1	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								

NOTE: Only rows designated as secondary antibody in the Layout tab can be selected in the Well Row drop-down menu.

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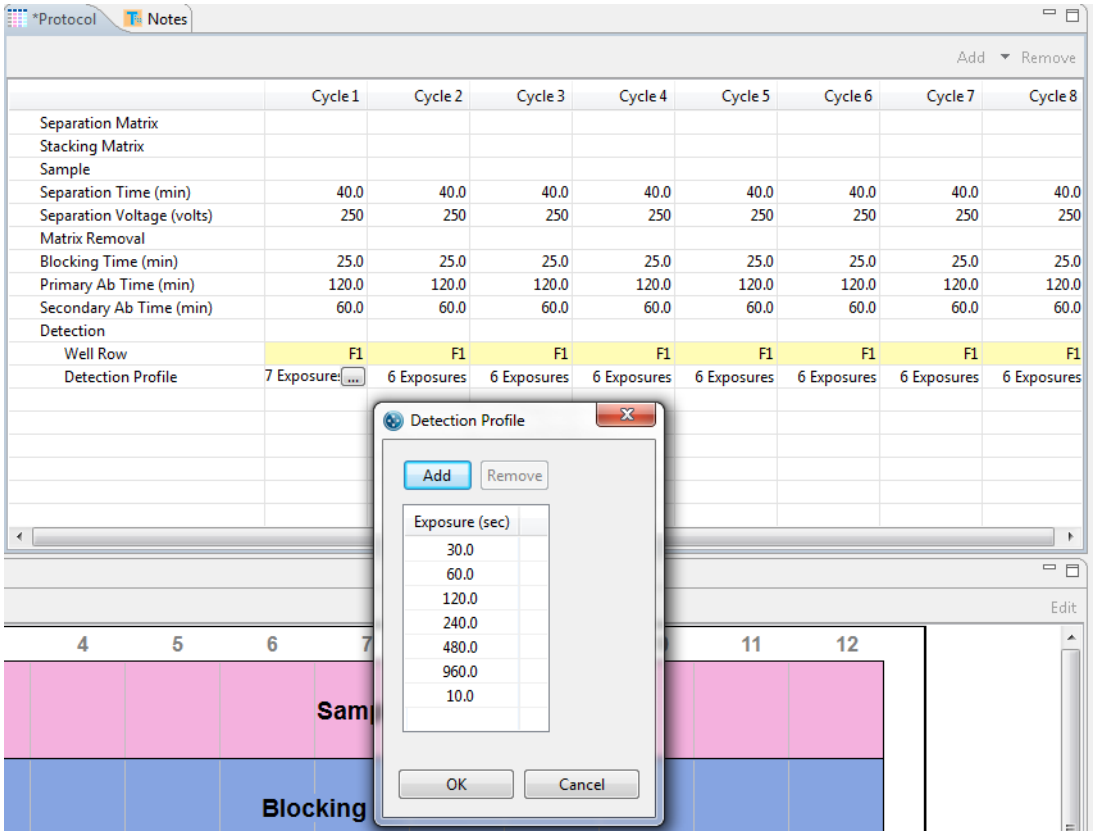
7. Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK.**



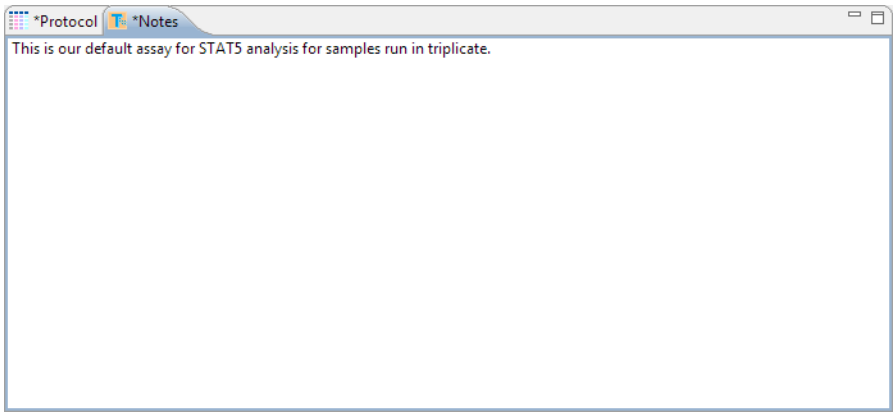
8. Modify any other protocol parameters as needed.

NOTES:
For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Step 4 - Add Assay Notes (Optional)

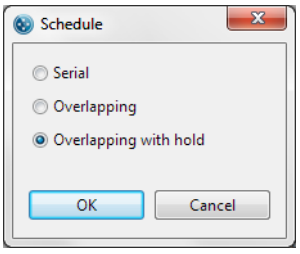
1. Click on the Notes tab.
2. Click in the pane and type any assay or protocol notes. This information will be stored with the assay and run data.



NOTE: When notes are being edited, an asterisk will appear in the Notes tab to indicate changes have been made and should be saved.

Step 5 - Select a Schedule (Optional)

Peggy can execute cycles serially or in parallel. To choose a schedule option, select **Edit** and click **Schedule**.



- **Serial** - Each cycle is executed in sequential order, and each cycle completes before the next one starts. Selecting this option result in the longest run times.
- **Overlapping** - Cycle steps are executed in parallel if timing permits shared use of the separation tray and robot. Each cycle will run through to completion once started. This option offers a reduction in overall run time without affecting the individual run time for each cycle.

- **Overlapping with Hold (default)** - Each cycle executes through the separate step in sequence and then enters a variable length hold step where capillaries remain in the incubation tray. More sample stability is provided with this option as proteins are 'locked' to the capillary wall and therefore more stable than samples on the assay plate. This option also results in the most significant reduction in overall run time.

NOTE: ProteinSimple recommends using the overlapping with hold option. For more information on when to use other schedule options, please contact ProteinSimple Technical Support.

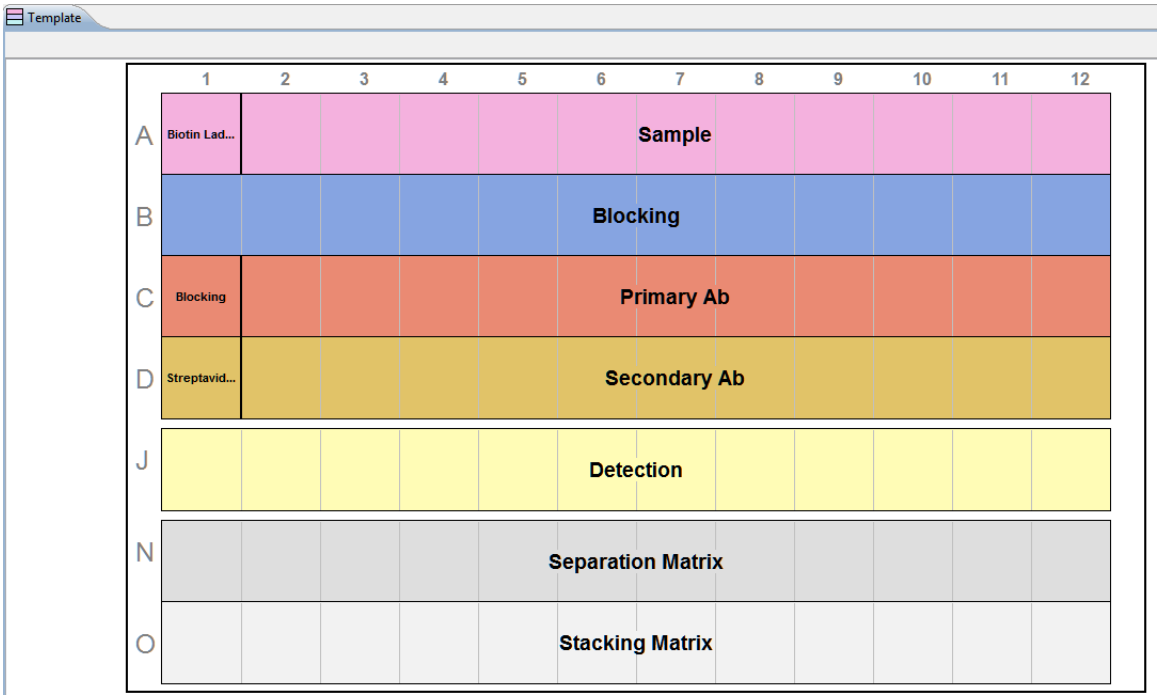
Step 6 - Add Assay Plate Annotations (Optional)

Custom reagent names, notes and annotations can be entered for individual rows and wells on the assay plate. This information will be stored with assay and run data. During post-run analysis, this information will be used to apply custom analysis settings to specific sample names, blocking reagent names, primary antibody names or sample and primary antibody attributes. This will be explained in more detail in "Compass Analysis Settings Overview" on page 219.

NOTE: Template pane information can also be added or updated after a run is complete.

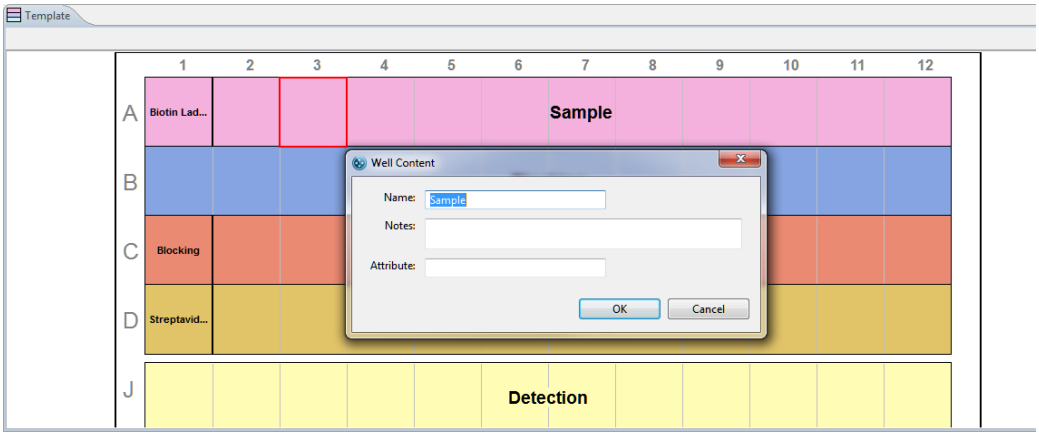
To enter annotations:

1. Click on the Template tab. The default annotations for reagent rows and individual wells on the assay plate will display:

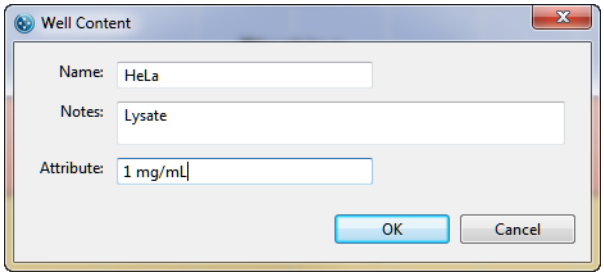


2. Change or add row and well annotations as needed. To do this:

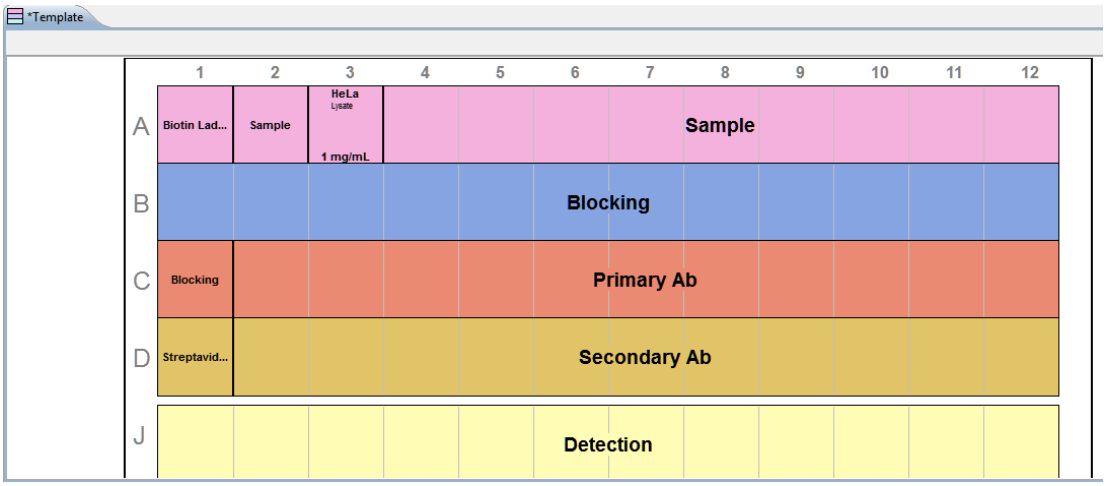
   a. **To enter annotations for a specific well** - Right click the well and select **Edit** or click **Edit** in the upper right corner of the pane or double click the selected well. The following box will display:



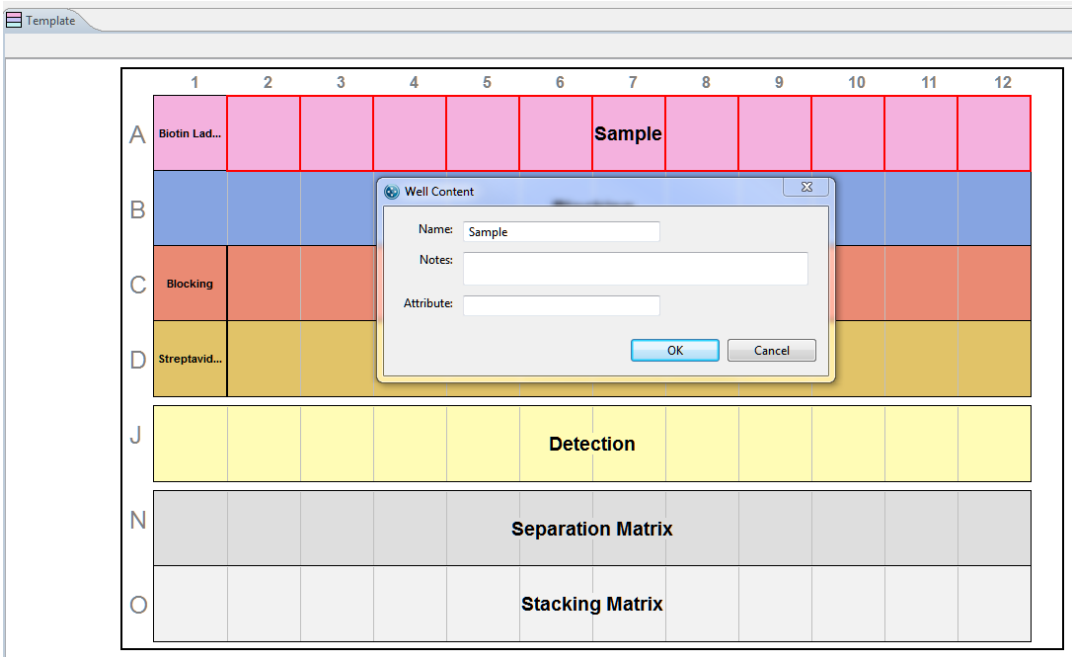
   Enter the reagent name, notes or specific attributes (for example, sample concentration or dilution ratio):



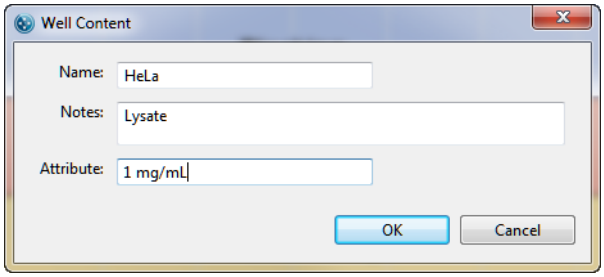
Click **OK**. The new information will display in the selected well:



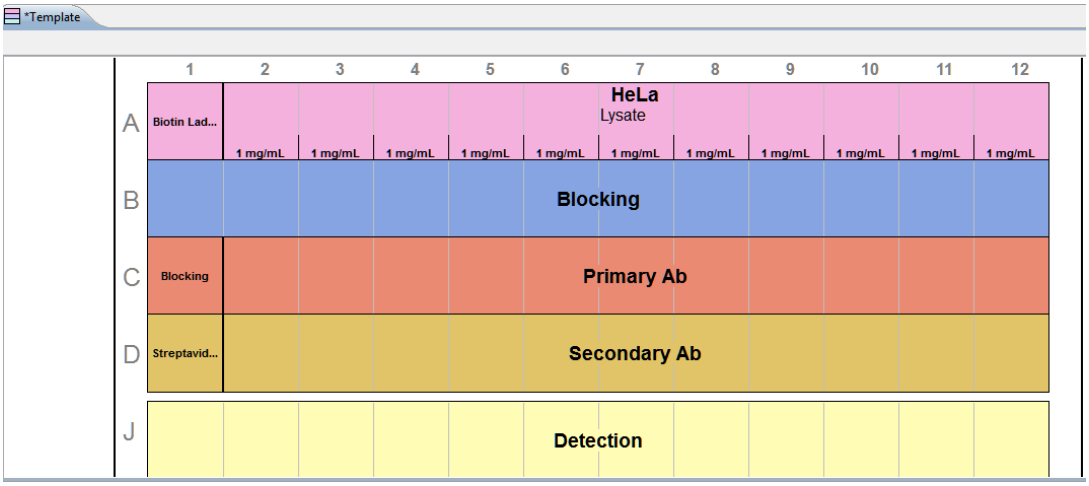
b. **To enter annotations for multiple wells or a row** - To select individual wells, press and hold the Control key and then select individual wells. To select a sequential set of wells or a full row, press and hold the Shift key, then select the first well and last well. Next, right click and select **Edit** or click **Edit** in the upper right corner of the pane. The following box will display:



Enter the reagent name, notes and specific attributes (for example, sample concentration or dilution ratio):



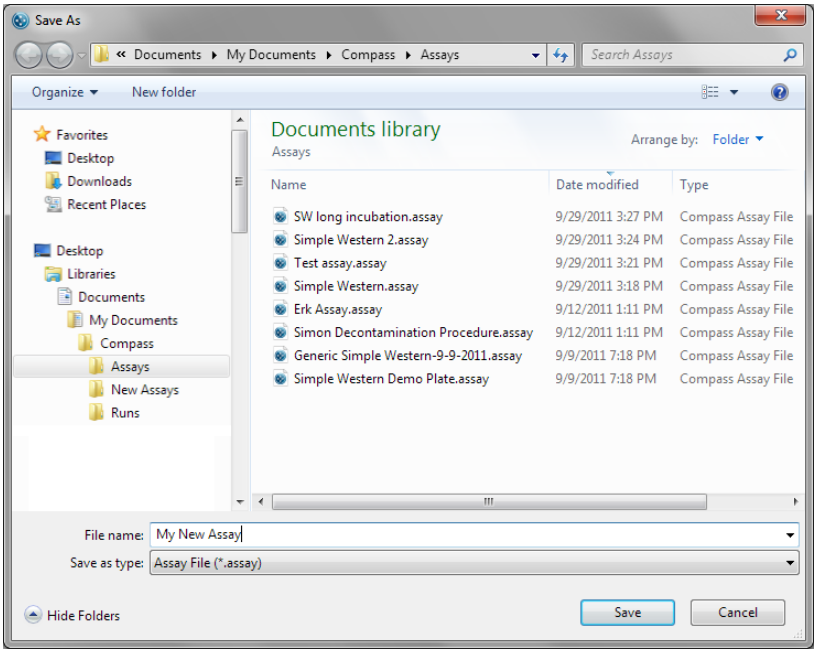
Click **OK**. The new information will display in the selected wells:



*NOTE: When annotations are being edited, an asterisk will appear in the **Template** tab to indicate changes have been made and should be saved. Only Name and Attribute will be used by Compass to annotate the data.*

Step 7 - Save the Assay

1. Select **File** from the main menu and click **Save As**. Enter the assay name and click **Save**.

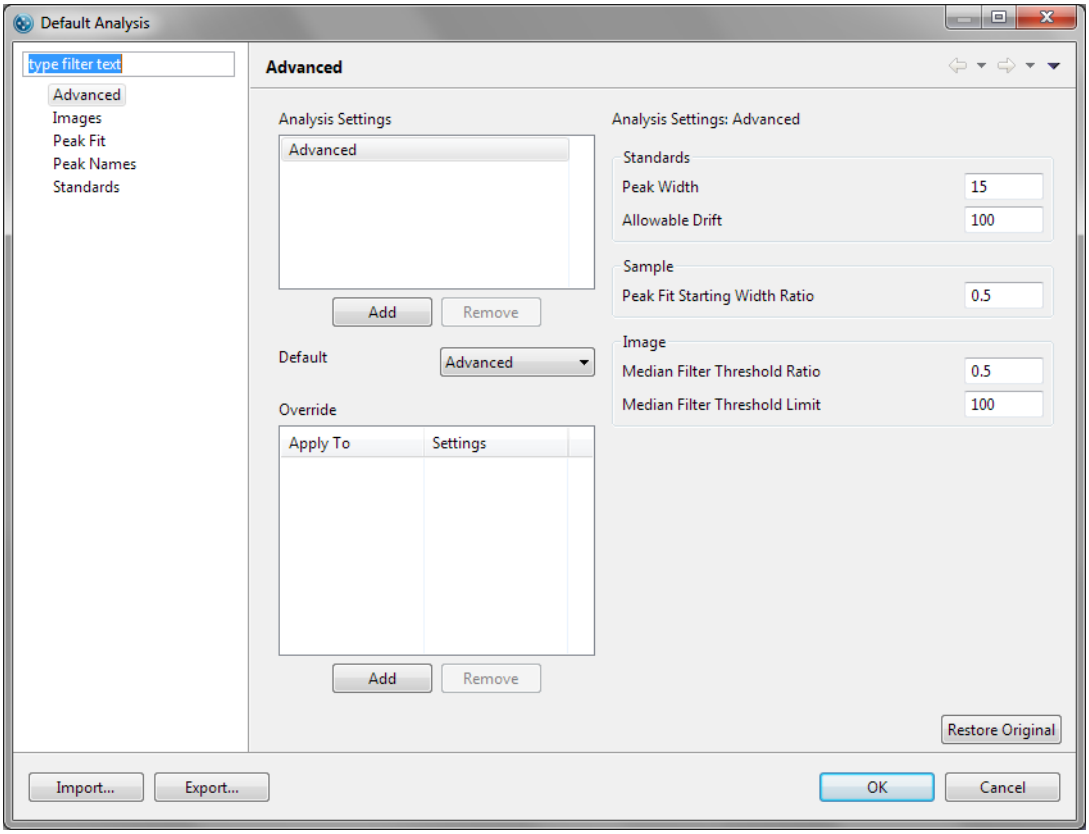


NOTE: New assays are saved in the Compass Assays directory.

Step 8 - Modify Default Analysis Parameters (Optional)

You can preset the parameters used to analyze run data generated with the assay.

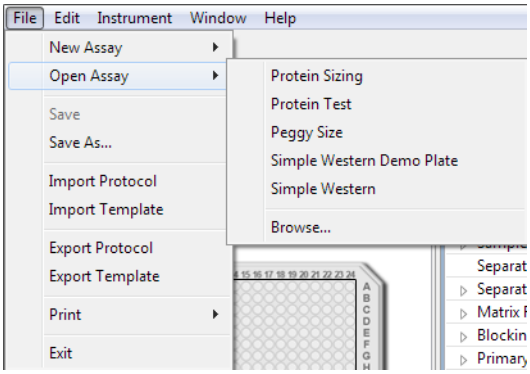
1. Select **Edit** from the main menu and click **Default Analysis**. The following screen will display:



2. ProteinSimple recommends using the default parameters for Simple Western assays. However, users can modify any parameters as needed, then click **OK**. For detailed information on analysis parameters, please refer to "Compass Analysis Settings Overview" on page 219.

Making Changes to an Existing Assay

1. Select **File** in the main menu and click **Open Assay**.

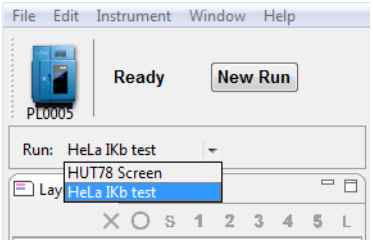


2. A list of the last assays opened will display. Select one of these assays or click **Browse** to open the assay folder and select a different assay.
3. Follow the steps in "Creating a New Assay" on page 25 to make changes and save the assay.

Switching Between Open Assays

If more than one run file is open, you can switch between viewing the assays for each run. To do this:

1. Click the down arrow in the run box.

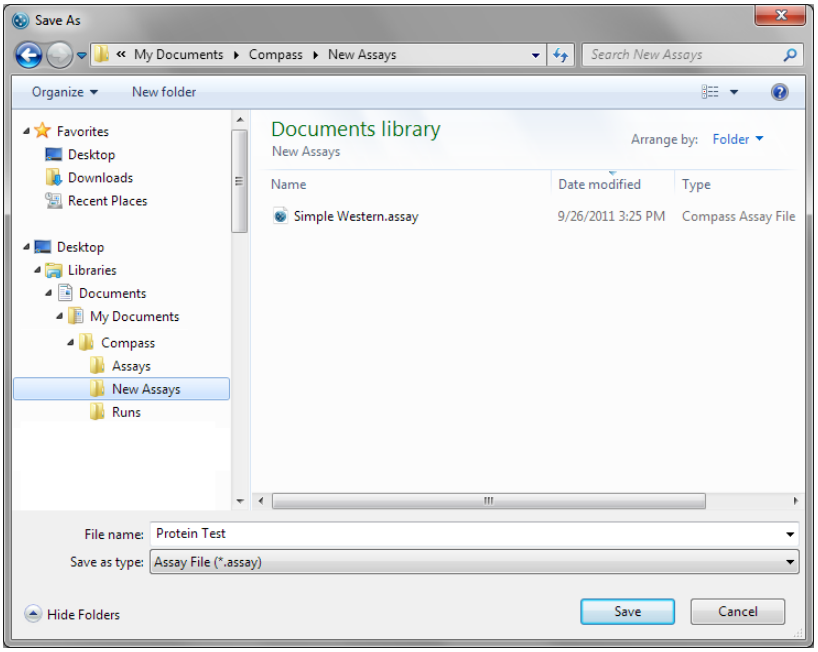


2. Select the run for the assay you want to view from the drop down list.

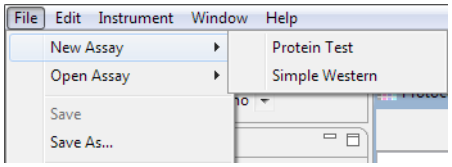
Creating a Template Assay

Users can create and save template assays that can be used as a starting point for creating new assays. To do this:

1. Select **File** in the main menu. Click **Open Assay** to open an existing assay or click **New Assay** to open an existing template assay.
2. Follow the steps in "Creating a New Assay" on page 25 to make changes to the assay.
3. When changes are complete, select **File** in the main menu and click **Save As**. Select the New Assays folder:

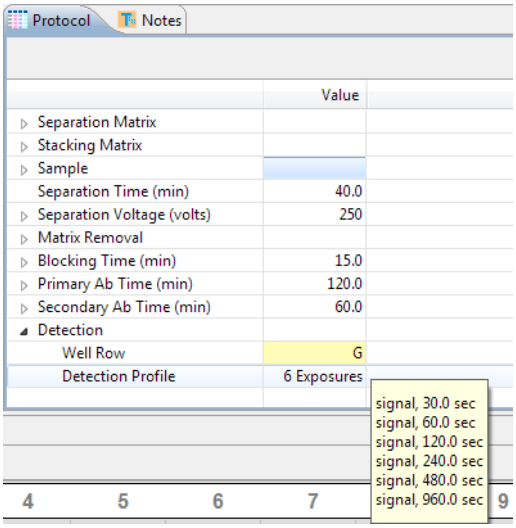


4. Type the name for the new template assay and click **Save**.
5. Select **File** in the main menu and click **New Assay**. The new template assay will now be available in the drop down list:



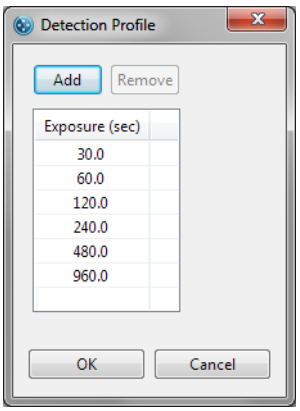
Viewing and Changing the Detection Exposures

To view the current detection profile, roll the cursor over the exposures cell in Protocol pane:



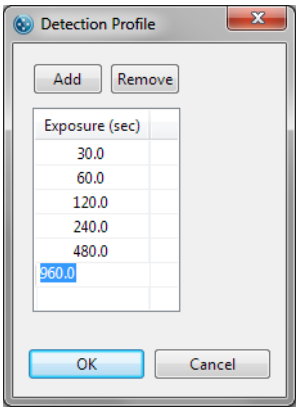
While ProteinSimple recommends using the default assay detection profile, users can change the profile, exposure times and exposure sequences if needed. To do this:

1. Select the exposures cell in the Protocol pane and click the button, or double-click in the cell. The following screen will display:



Each row represents an individual exposure that will be taken during the run.

a. **To change an existing exposure time** - Click in the exposure cell and enter a new time in seconds:



b. **To delete an existing exposure** - Select a type or exposure cell and click **Remove**.

c. **To add a new exposure** - Select **Add**. A new exposure will be added to the end of the list. Click in the exposure cell and enter an exposure time in seconds.

2. Click **OK** to save and exit.

Copying Protocols and Templates

The steps and parameters in the Protocol pane can be copied and d into other documents, as can the graphic image of the annotations in the Template pane.

Copying an Assay Protocol

1. Click on the Protocol tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Word, Excel, etc). Right click in the document and select. All assay protocol steps and parameters will be copied into the document as a list in the same format shown in the Protocol pane.

Copying an Assay Template

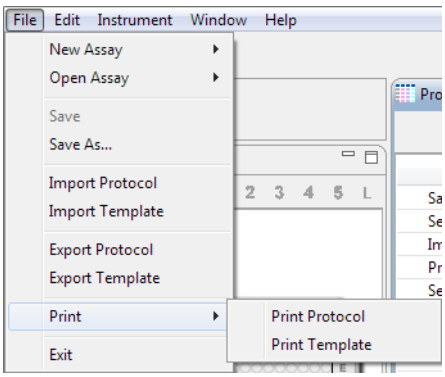
1. Click on the Template tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Word, Excel, etc). Right click in the document and select. The assay template will be copied into the document as an image, exactly as it is shown in the Template pane.

Printing Protocols and Templates

The information in the Protocol pane can be printed, as can a graphic image of the information in the Template pane.

Printing an Assay Protocol

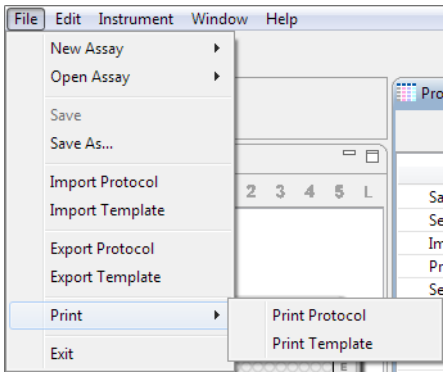
1. Click on the Protocol tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Protocol**.



All assay protocol steps and parameters will be printed as a list in the same format shown in the Protocol pane.

Printing an Assay Template

1. Click on the Template tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Template**.



The assay template will print as an image, exactly as it is shown in the Template pane.

Importing and Exporting Protocols and Templates

The assay protocol in an open assay or run file can be exported as a separate file, as can the assay template annotation information. This allows the same assay protocol and template information to be imported into another assay at a later time, rather than having to re-enter assay protocol and template information manually.

Importing an Assay Protocol

NOTE: Importing an assay protocol imports information into the Protocol pane only.

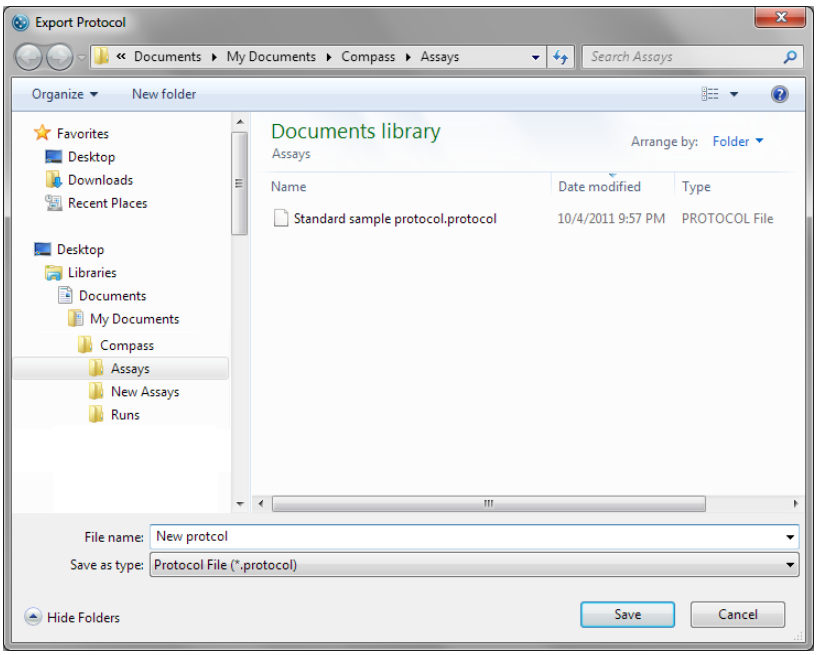
---

1. Open the assay you want to import the assay protocol in to.
2. Select **File** in the main menu and click **Import Protocol**.
3. Select a protocol file (*.protocol) and click **OK**. The imported information will display in the Protocol pane.

Exporting an Assay Protocol

NOTE: Exporting an assay protocol exports information in the Protocol pane only.

1. Open the assay you want to export the assay protocol from.
2. Select **File** in the main menu and click **Export Protocol**. The following window displays:



3. The default directory is Compass/Assays. Change the directory if needed.
4. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file.

Importing an Assay Template

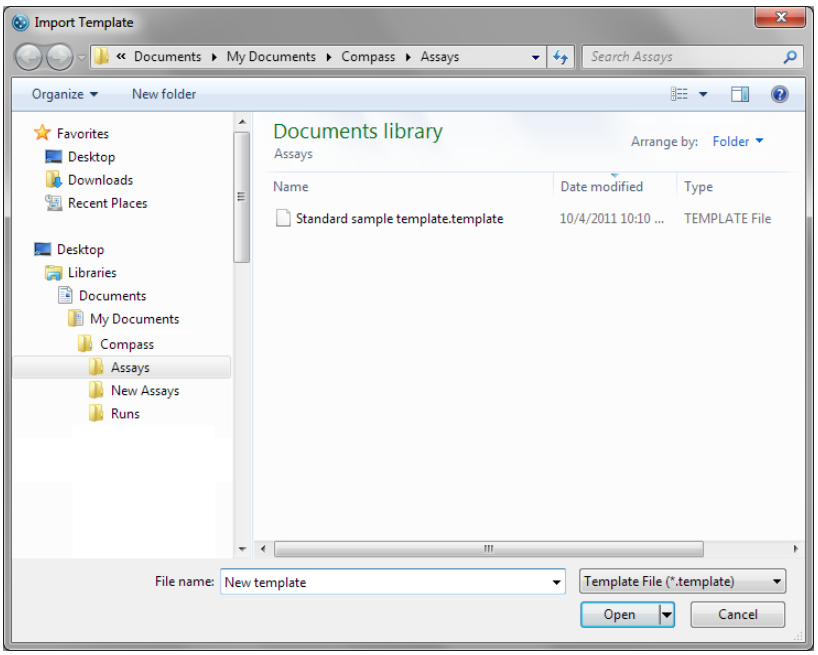
NOTE: Importing an assay template imports information into the Template pane only.

1. Open the assay you want to import the assay template in to.
2. Select **File** in the main menu and click **Import Template**.
3. Select a template file (*.template) and click **OK**. The imported information will display in the Template pane.

Exporting an Assay Template

NOTE: Exporting an assay template exports information in the Template pane only.

1. Open the assay you want to export the assay template from.
2. Select **File** in the main menu and click **Export Template**. The following window displays:



3. The default directory will be Compass/Assays. Change the directory if needed.
4. Enter a template name and click **Save**. The template will be saved as a *.template file.

Chapter 3:

Running a Size Assay on Peggy

Chapter Overview

- Starting a Run
- Stopping a Run

Starting a Run

Step 1 - Get Ready

1. Open Compass software.
2. Prepare instrument: empty waste, refill water and add a new manifold sponge.
3. Create or open desired assay file.
4. Prepare assay plate following the procedure described in the product insert.

IMPORTANT
To prevent well evaporation and ensure best results, keep a lid on the assay plate until ready to use.

---

5. While plate is spinning, add Wash, Running and Matrix Removal Buffers to resource tray cups. Place capillary box in the designated resource tray position.

IMPORTANT
Capillaries are light sensitive. Keep the cover on the box until you are ready to transfer the capillary box to the resource tray.

---

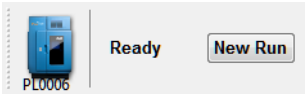
6. Place assay plate into the sample tray of the instrument and press **Start**.

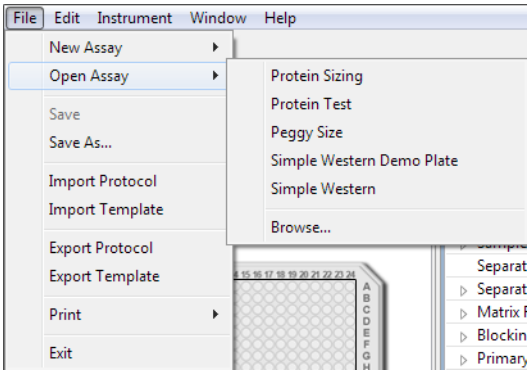
Step 2 - Start the Run

1. You can start a run in one of two ways depending on what button is displayed in the status bar.

   NEW RUN BUTTON- click **New Run**.

a.Select **File** in the main menu and click **Open Assay**.



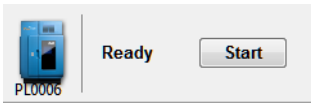


b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

c. Alternatively, choose **New Assay** and select **Peggy Size** to get the default Peggy assay conditions.

d. The **Start** button will display.

START BUTTON - this indicates an assay is already loaded.



a. Go to the Assay screen and verify this is the assay you want to use. If not, select **File** in the main menu, click **Open Assay**, and select another assay.

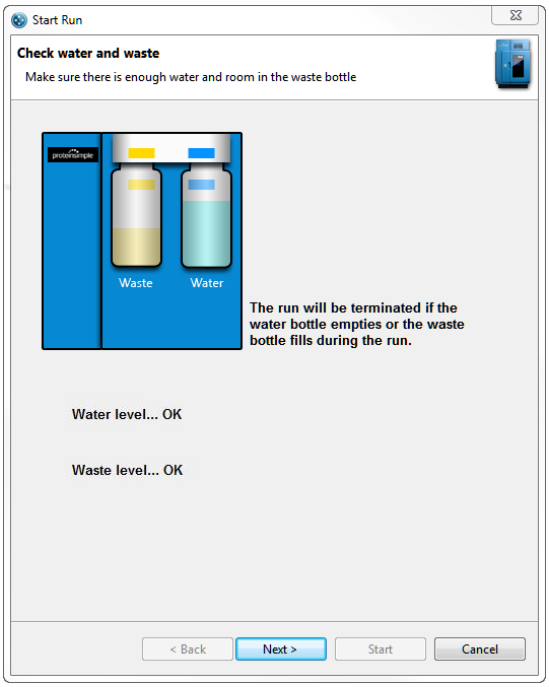
2. Click **Start**. This will launch the **Start Run Wizard**.

---

*NOTE: If the manifold was not cleaned prior to starting the run, a message indicating this will display. If this occurs, click **Yes** to cancel the run and perform the manifold cleaning.*

---

3. **Page 1: Check Water and Waste.** The fluid levels in the accessory module bottles will be checked by the software. If the levels in both bottles will allow Peggy to complete the run, the wizard screen will display **Water Level OK** and **Waste Level OK** messages. Click **Next** to proceed.

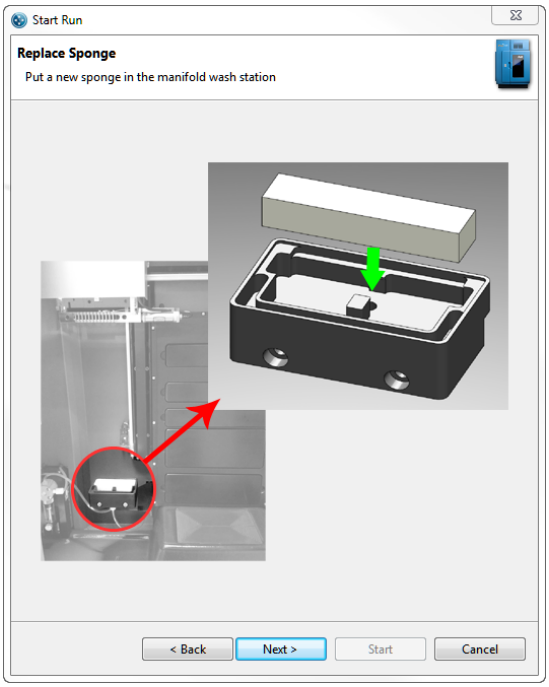

Start Run
Check water and waste
Make sure there is enough water and room in the waste bottle
Waste
Water
The run will be terminated if the water bottle empties or the waste bottle fills during the run.
Water level... OK
Waste level... OK
< Back
Next >
Start
Cancel

---

*NOTE: If the waste level is too high or the water level is too low to complete the run, messages to indicate one or both will be presented in this screen. If this occurs, fill or empty each bottle as indicated using the procedures outlined in the Peggy User Guide. When this is complete, the error status will be automatically updated and allow the **Start Run Wizard** to proceed.*

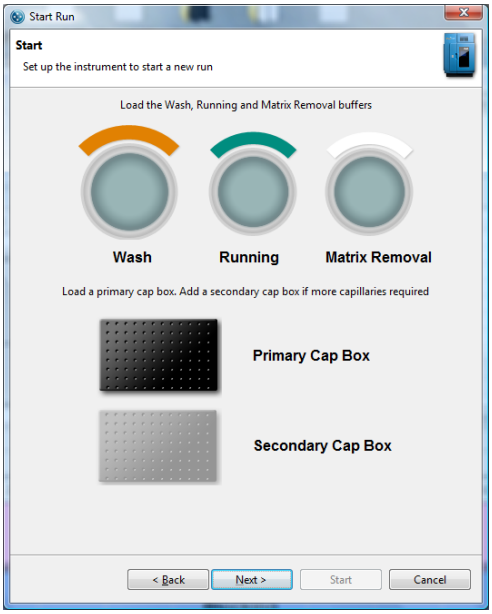
---

4. **Page 2: Replace Sponge.** A new sponge should be used each time a new experimental run is started. Discard the old sponge and put a new sponge in the manifold wash station.

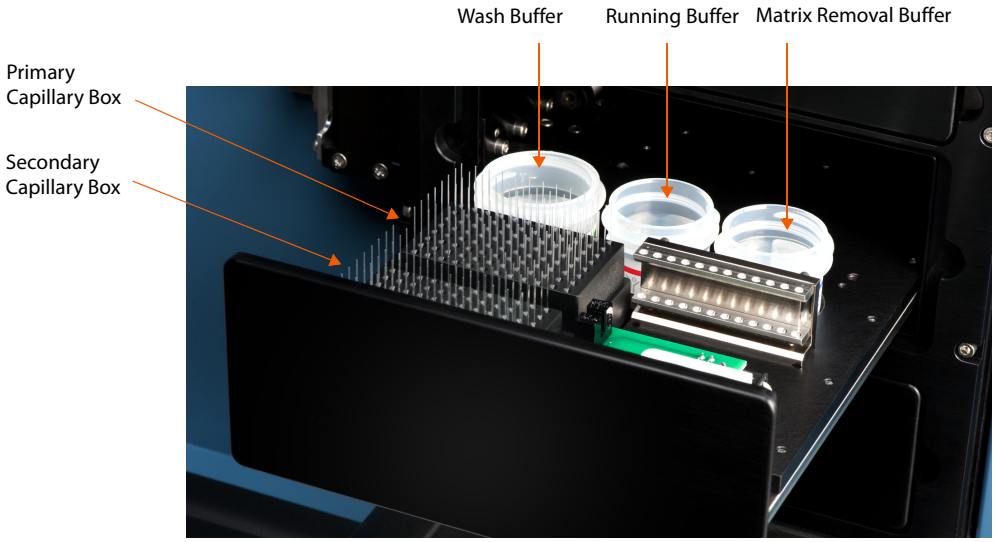


5. **Page 3: Start.** The resource tray will automatically open. Fill the Wash Buffer, Running Buffer, and Matrix Removal Buffer cups and insert the capillary box in the primary capillary box position. Add a secondary capillary box if more capillaries are required. Click **Next** when finished. The resource tray will close.

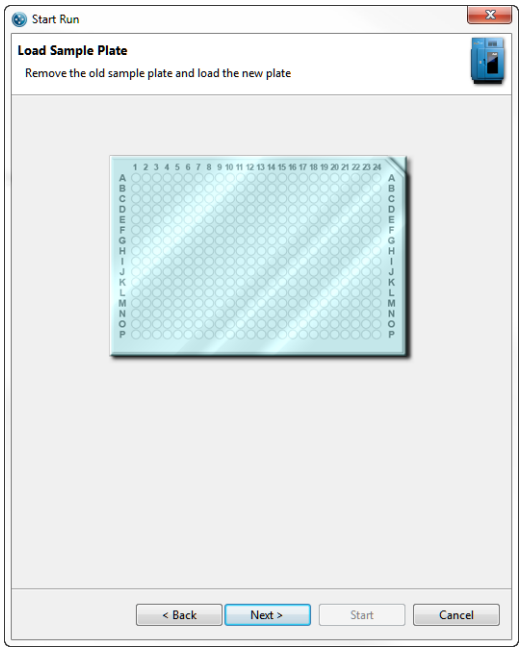
NOTE: Make sure enough capillaries are loaded to complete the run. A capillary box should be inserted in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is depleted, Peggy will automatically move to the secondary box. Discard leftover Running Buffer, Wash Buffer and Matrix Removal Buffer prior to refilling bottles for new run.

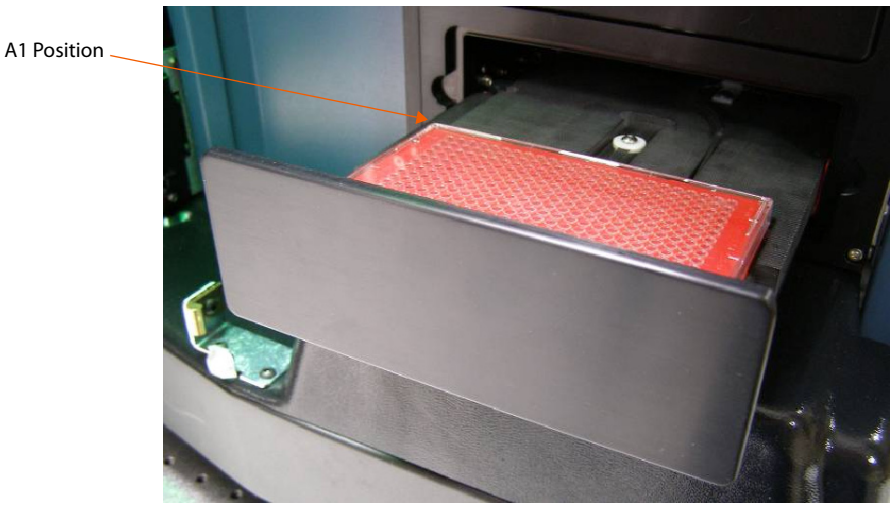


NOTE: You an also refer to the labels on the resource tray for proper insertion of reagents.



6. **Page 4: Load Sample Plate.** The sample tray will automatically open. Place the lidded 384-well plate on the cooling block in the tray, ensuring that the A1 well position is aligned with the upper left corner of the cold block. When this is complete, click **Next**. The sample tray will close.



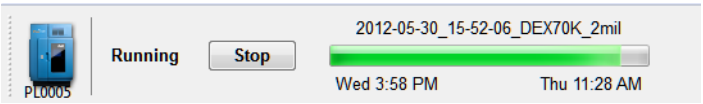


NOTES:
Peggy requires that plate lids be used on sample plates. If a lid is not detected, a message will be displayed in the Start Run Wizard. Compass software will reopen the sample tray to allow the user to insert a lid.

When inserting the sample plate, ensure that the plate is firmly seated and level on the cold block. Plates that are not level can interfere with the movement of the sample tray.

7. **Page 5: Data File.** The data file name will automatically default to the assay name appended with the current date and time. To change the file name, begin typing in the text box. To change the directory where the data file will be stored, click **Browse**:

Click **Start** to begin the run. Instrument status will change to running, and the stop button and progress bar will display:



The run will continue until complete (~14-19 hours depending on the assay).

Step 3 - Post-Run Procedures

1. Empty the capillary discard tray.
2. Remove the assay plate.
3. Dispose of the assay plate and capillaries. Disposal will depend on the samples that have been assayed. If sample origins are unknown, ProteinSimple recommends that used capillaries and plates be disposed of in biohazard waste.

!WARNING! SHARPS HAZARD

The capillaries may present a potential sharps hazard. Dispose of used capillaries in biomedical waste sharps containers.

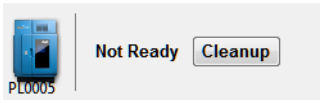
!WARNING! BIOHAZARD

Samples and waste bottle contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations. Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste vial before you store, handle, or dispose of chemical waste.

Stopping a Run

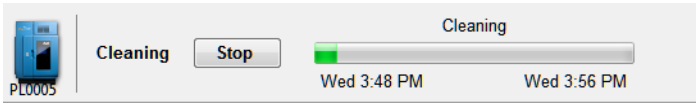
1. To stop a run, click **Stop**. When the run stops, instrument status will go to Not Ready and a Cleanup button displays:



NOTE: If a run is stopped prior to completion, Peggy must perform a cleaning protocol to remove used capillaries from system trays. This is required to prepare the system for the next run.

---

2. Click **Cleanup**.



Allow Peggy to complete the cleaning protocol which takes about ten minutes. When complete, instrument status will change to Ready and a new run can be started.

Chapter 4:

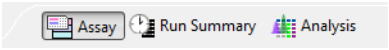
Charge Assays

Chapter Overview

- Assay Screen Overview
- Reagent Color Coding
- Opening an Assay
- Creating a New Assay
- Making Changes to an Existing Assay
- Switching Between Open Assays
- Creating a Template Assay
- Viewing and Changing the Detection Exposures
- Copying Protocols and Templates
- Printing Protocols and Templates
- Importing and Exporting Protocols and Templates

Assay Screen Overview

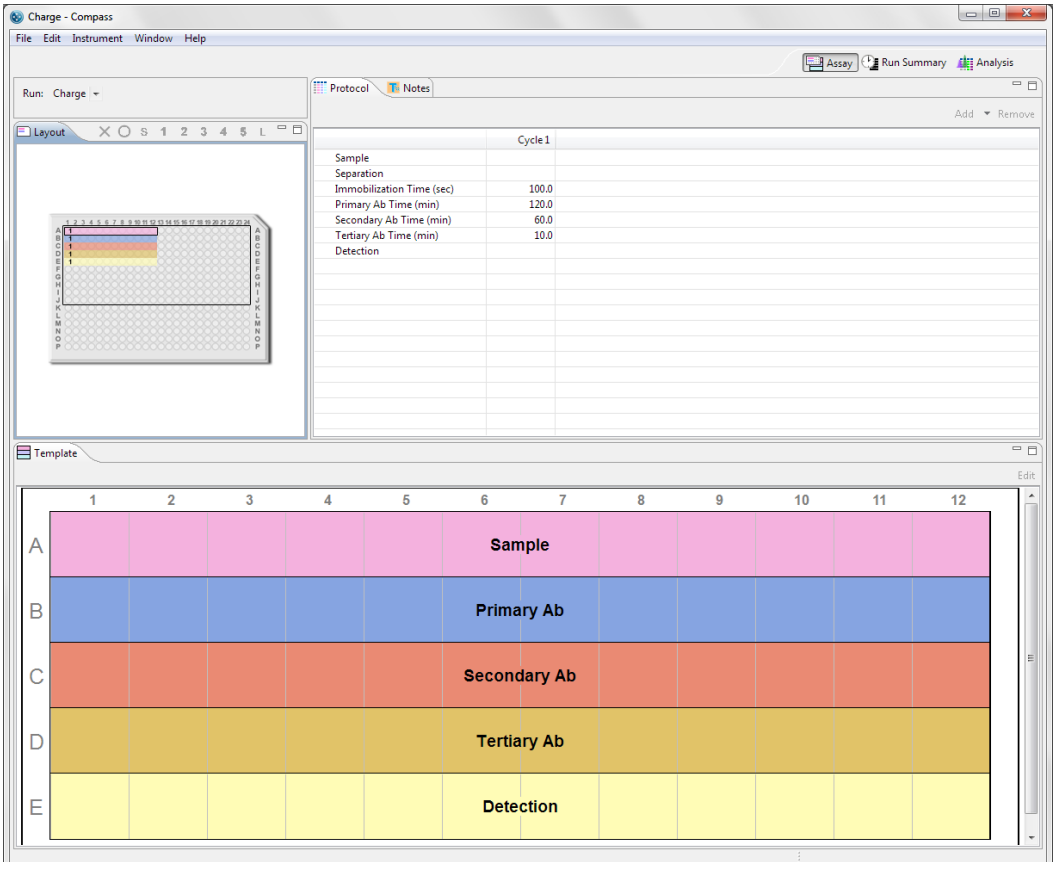
The Assay screen is used to create, view, and edit assays. To access this screen, click **Assay** in the screen tab:



Assay Screen Panes

The Assay screen has four panes:

- **Layout** - Displays a map of the assay plate and shows where assay reagents will be located.
- **Protocol** - Lists individual assay protocol steps and parameters that Peggy will execute for each of the 12 capillaries simultaneously.
- **Notes** - Lets you enter specific assay information that is saved with the assay and can be used for future reference.
- **Template** - Enter annotations for the individual well and row reagents in the assay plate.



Software Menus Active in the Assay Screen

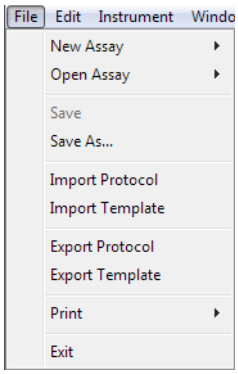
The following software menus are available:

- File
- Edit
- Instrument (when Compass is connected to Peggy)
- Window
- Help

The File and Edit menu options specific to the Assay screen are described next.

File Menu

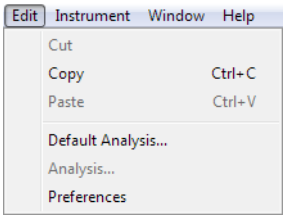
The following File menu options are active:



- **New Assay** - Creates a new assay from a starter template.
- **Open Assay** - Opens an existing assay.
- **Save** - Saves the open assay.
- **Save As** - Saves the open assay under a different file name.
- **Import Protocol** - Imports a saved protocol file into an assay.
- **Import Template** - Imports a saved template file into an assay.
- **Export Protocol** - Exports the current protocol file for future use.
- **Export Template** - Exports the current template file for future use.
- **Print** - Prints the information in the Protocol or Template panes.
- **Exit** - Closes Compass.

Edit Menu

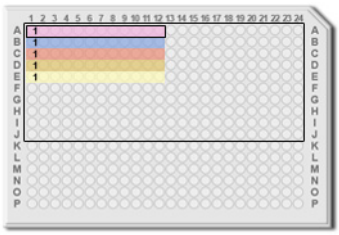
The following Edit menu options are active:



- **Copy** - Copies the information in the Protocol or Template panes into other documents.
- **Default Analysis** - Displays the default settings that will be used to analyze the run data generated with an assay.
- **Analysis** - Not active in this screen.
- **Preferences** - Set and save custom preferences for data export, plot colors in the graph and Peggy's Twitter settings. See Chapter 10, *"Setting Custom User Preferences"* for more information.

Reagent Color Coding

The Assay screen uses color coding to identify various assay reagents in all panes. The Layout pane is shown in the following example:

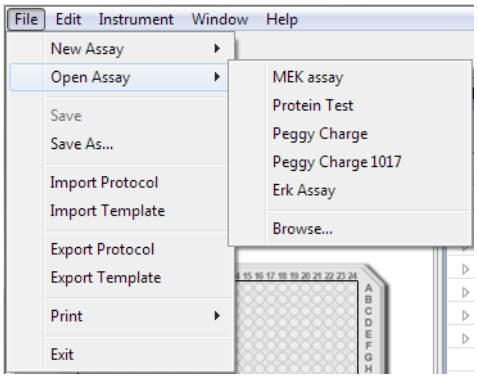


- **Pink** - Samples
- **Blue** - Primary antibody
- **Orange** - Tertiary antibody
- **Gold** - Secondary HRP conjugate
- **Yellow** - Luminol/Peroxide mix

Opening an Assay

To open an existing assay:

1. Select **File** in the main menu and click **Open Assay**.



2. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

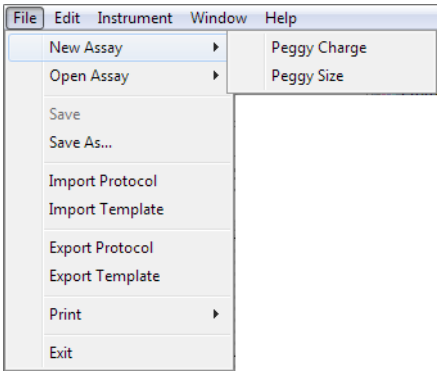
Creating a New Assay

To create a new assay, ProteinSimple recommends using the Peggy template assay and modifying from there as needed.

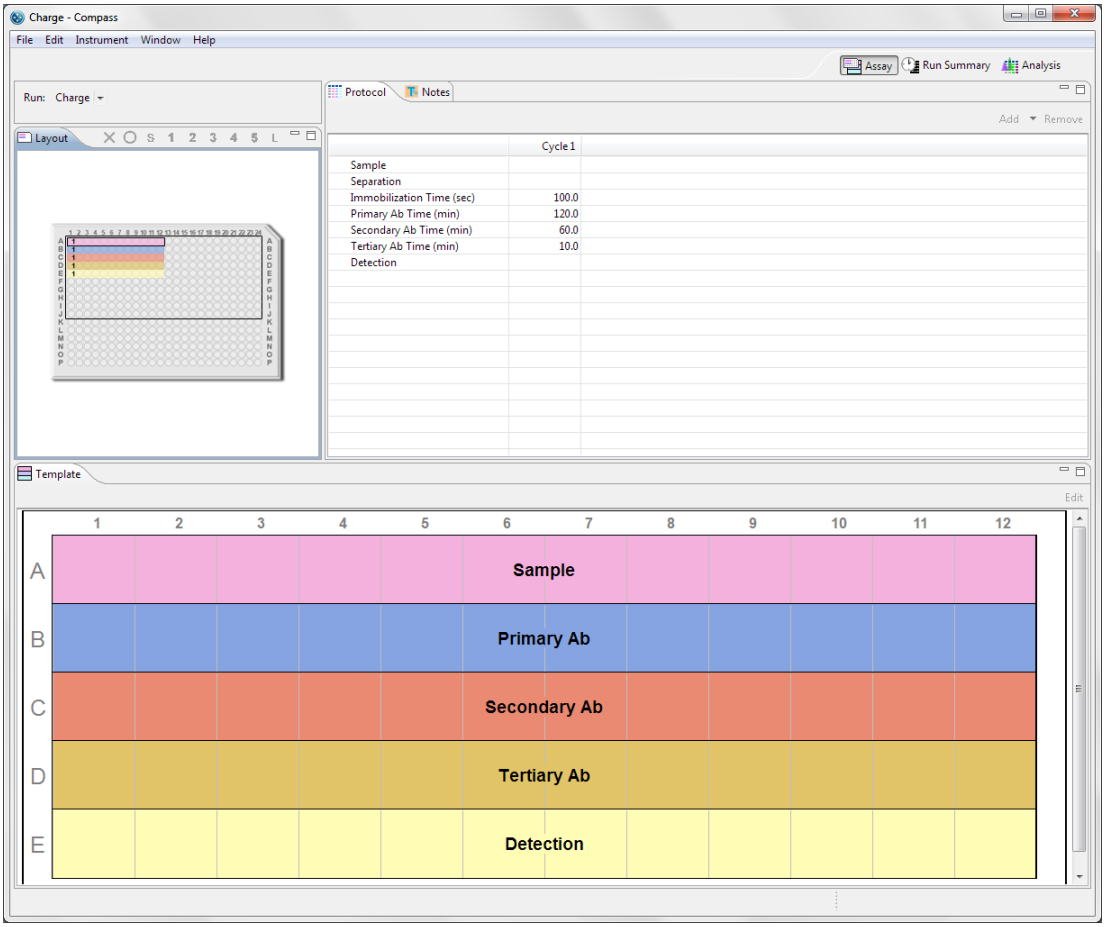
Step 1 - Open a Template Assay

1. Select **File** in the main menu and click **New Assay**:

T



2. A list of template assays that can be used as a starting point for new assays will display. Click **Peggy Charge**. The Peggy template assay and default settings will display in the Assay screen:

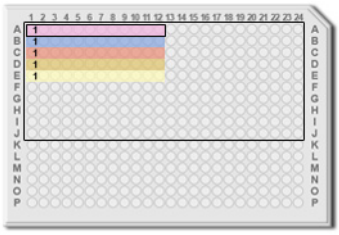


Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. This pane displays the default row locations of where each reagent should be placed on the assay plate.

NOTE: Up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.

- **Row A** - Samples

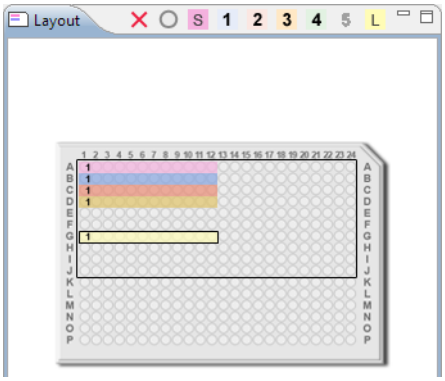


- **Row B** - Primary Antibody
- **Row C**- Secondary Antibody
- **Row D** - Tertiary Antibody (optional)
- **Row E** - Luminol-S/Peroxide mix

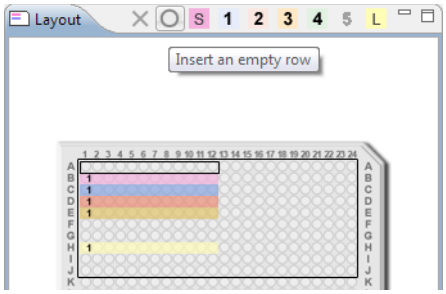
NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

---

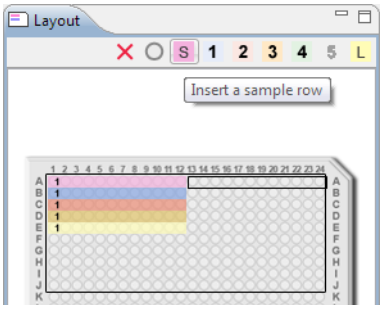
2. If needed, well assignments can be modified as described below. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
   - **To move a reagent row to another location** - Click the row in the **Layout** pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.



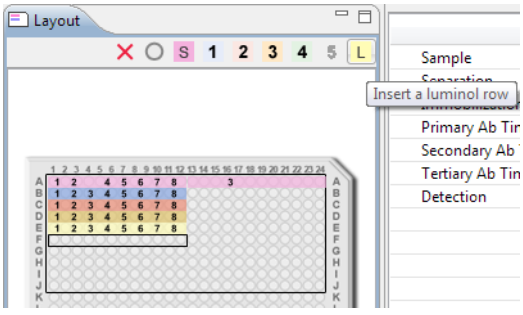
   - **To insert a new row** - Click the row below where the new one should be inserted, then click **Insert an empty row** (circle icon) in the **Layout** pane toolbar. A new row will be inserted above the selected row.



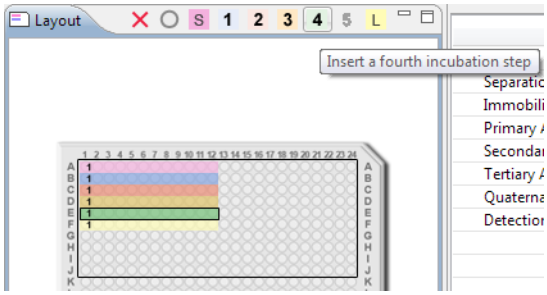
- **To insert a sample row -** Click an empty row or the row below where the new sample row should be inserted, then click **Insert a sample row** (S icon) in the **Layout** pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.



- **To insert a luminol row -** Click an empty row or the row below where the new luminol row should be inserted, then click **Insert a luminol row** (L icon) in the **Layout** pane toolbar. A new luminol row will be added in the empty row or inserted above the selected row.



- **To insert a fourth or fifth incubation reagent** - Click an empty row or the row below where the new incubation reagent should be inserted, then click **Insert a 4th incubation step** (4 icon) in the **Layout** pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.

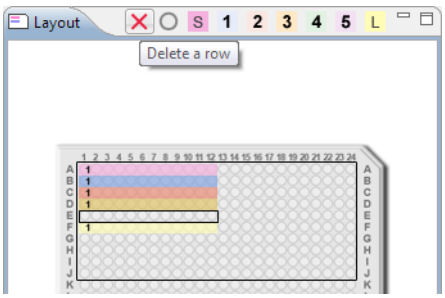


A fifth incubation reagent can now be added by repeating the above and clicking **Insert a 5th incubation step** (5 icon) instead.

NOTES:
When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

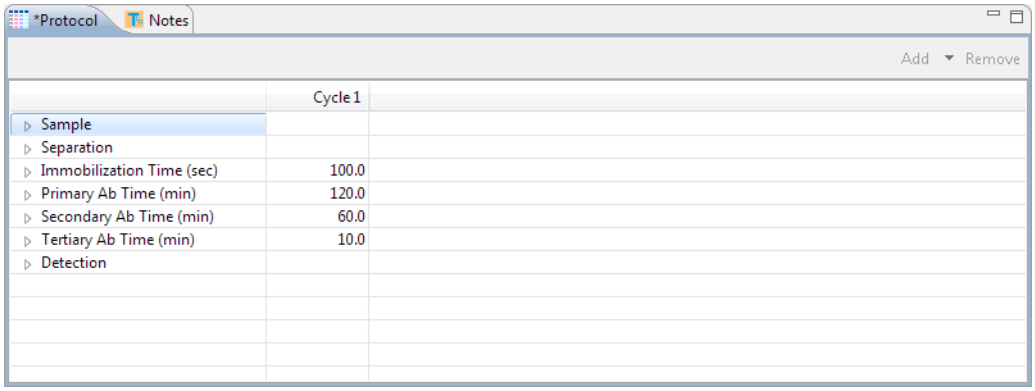
- **To delete a row** - Click the row to be deleted, then click **Delete** (red x icon) in the toolbar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.



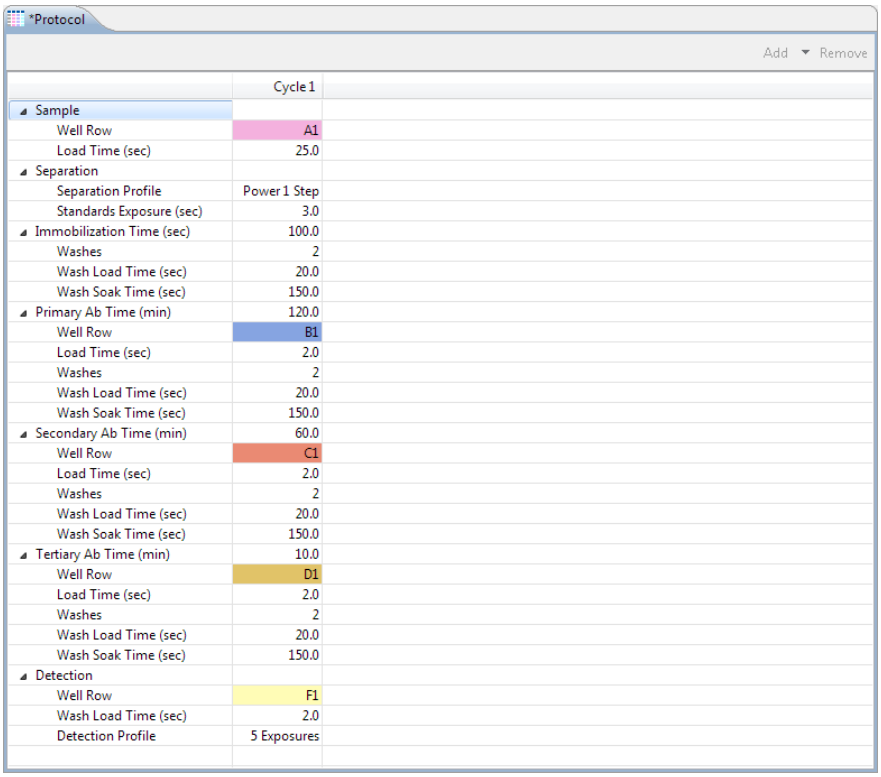
NOTE: Samples, antibodies and blocking buffer can be dispensed in Rows A-J and in columns 1-12 or 13-24. Rows K-P cannot be used for assay reagents.

Step 3 - Modifying the Assay Protocol (Optional)

1. Click on the Protocol tab. This pane displays the individual steps of the assay protocol and allows you to change parameters as needed. When creating a new assay, a default protocol will display which automatically assigns all reagent locations for Cycle 1:



Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. ProteinSimple recommends using the default protocol settings for Peggy assays. An expanded list of the default protocol step parameters is shown:

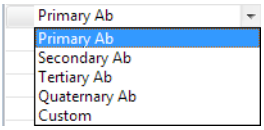


	Cycle 1
◢ Sample	
Well Row	A1
Load Time (sec)	25.0
◢ Separation	
Separation Profile	Power 1 Step
Standards Exposure (sec)	3.0
◢ Immobilization Time (sec)	100.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
◢ Primary Ab Time (min)	120.0
Well Row	B1
Load Time (sec)	2.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
◢ Secondary Ab Time (min)	60.0
Well Row	C1
Load Time (sec)	2.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
◢ Tertiary Ab Time (min)	10.0
Well Row	D1
Load Time (sec)	2.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
◢ Detection	
Well Row	F1
Wash Load Time (sec)	2.0
Detection Profile	5 Exposures

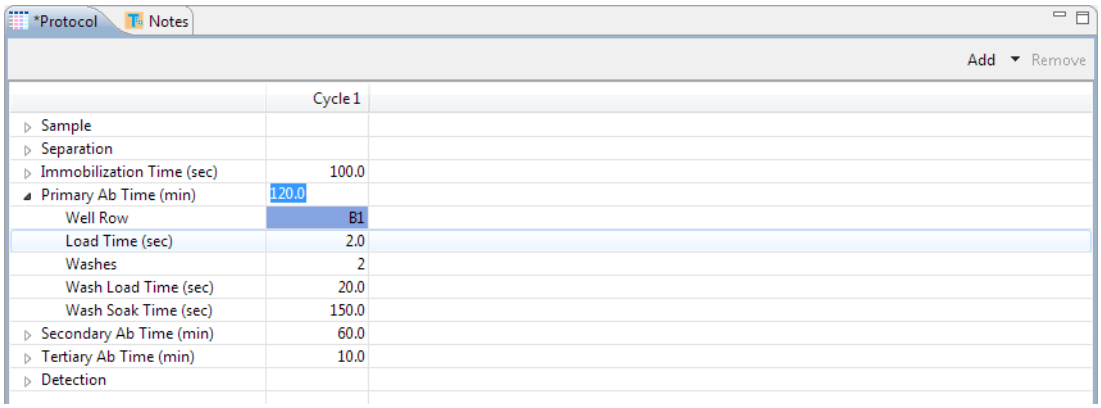
2. Five incubation steps are allowed per protocol. Users can select the type of incubation for each step. The available incubation types and their default Simple Western use is as follows:

   - **First incubation** - Primary antibody
   - **Second incubation** - Secondary antibody
   - **Third incubation** - User defined (tertiary antibody)
   - **Fourth incubation** - User defined (quaternary antibody)
   - **Fifth incubation** - User defined (custom)

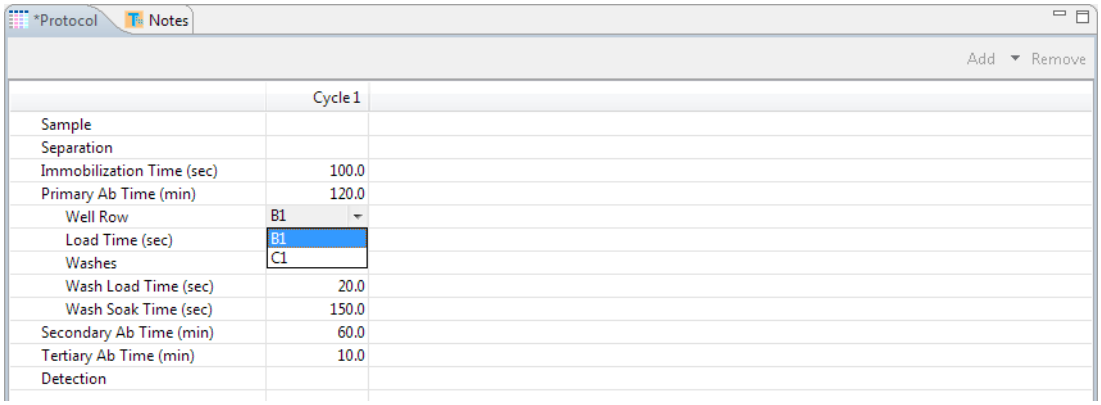
   To change the type, click the incubation step name and select an option from the drop down list.



3. If needed, change the primary incubation time. To do this, click the cell in the value column next to Primary Ab Time (min) and enter a new value in minutes:



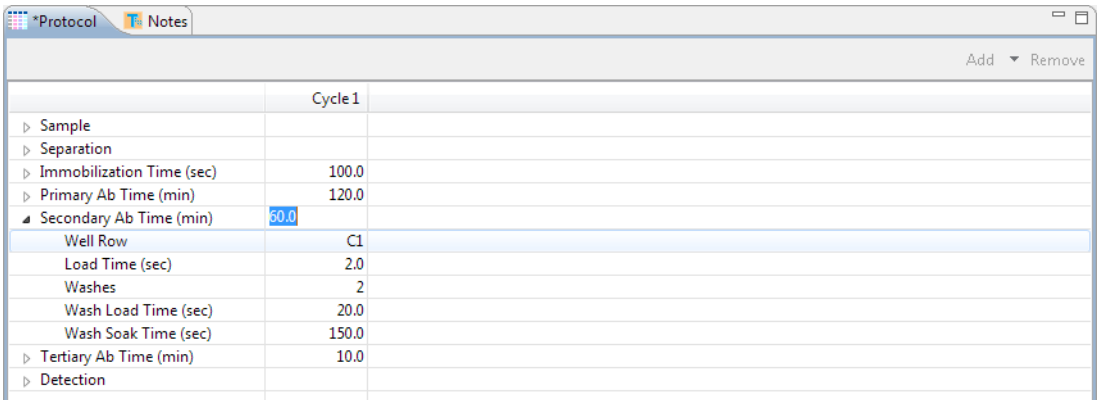
4. If needed, change the primary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:



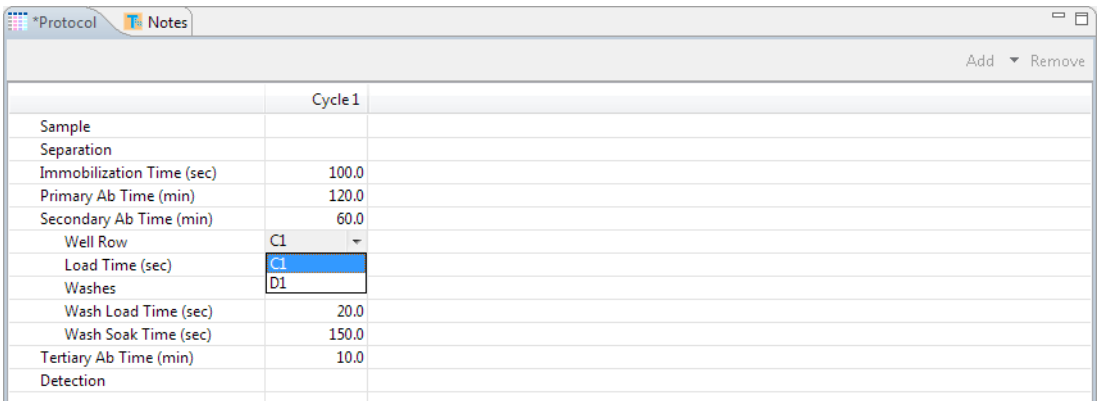
NOTE: Only rows designated as primary antibody in the Layout tab can be selected in the Well Row drop-down menu.

---

5. If needed, change the secondary incubation time. To do this, click the cell in the value column next to Secondary Ab Time (min) and enter a new value in minutes:



6. If needed, change the secondary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:



NOTE: Only rows designated as secondary antibody in the Layout tab can be selected in the Well Row drop-down menu.

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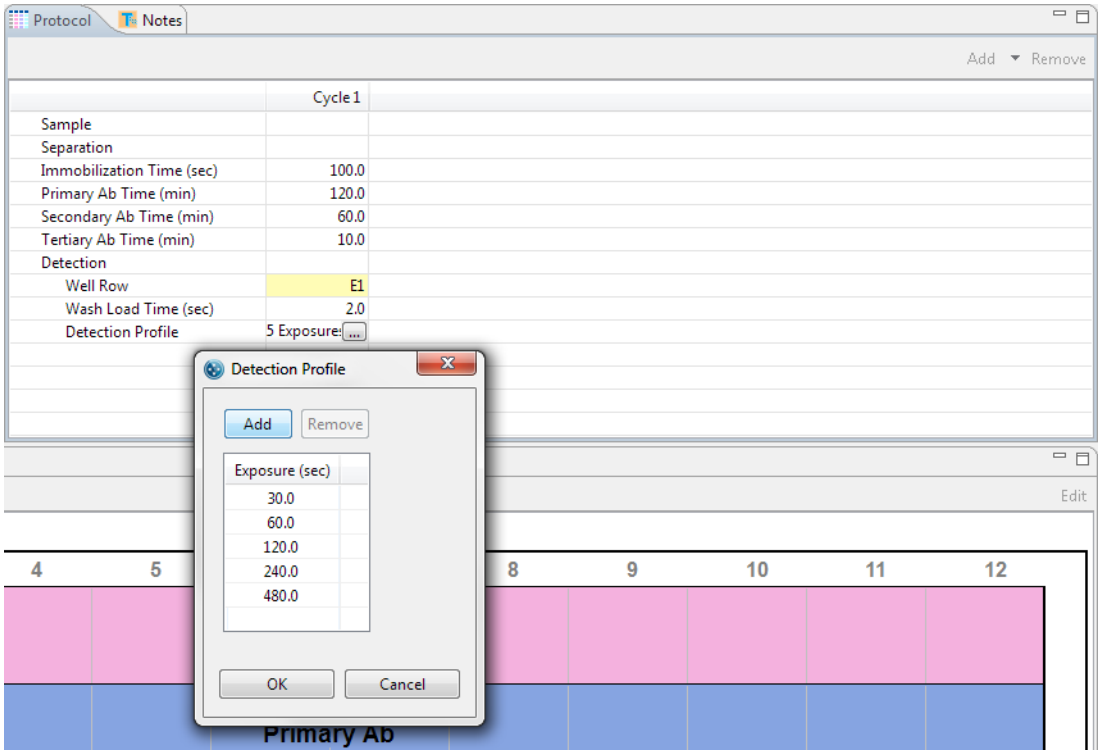
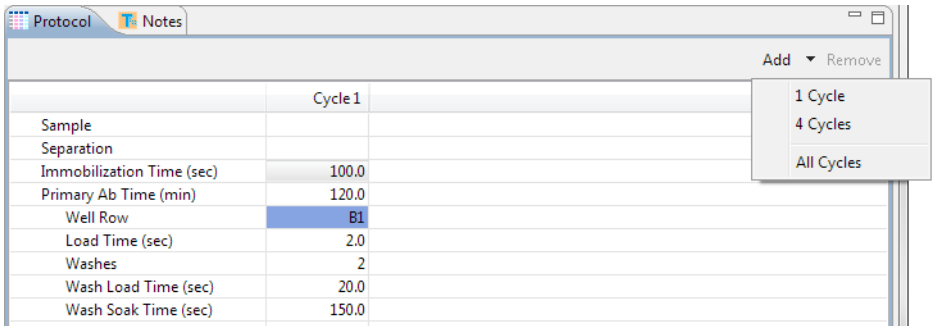
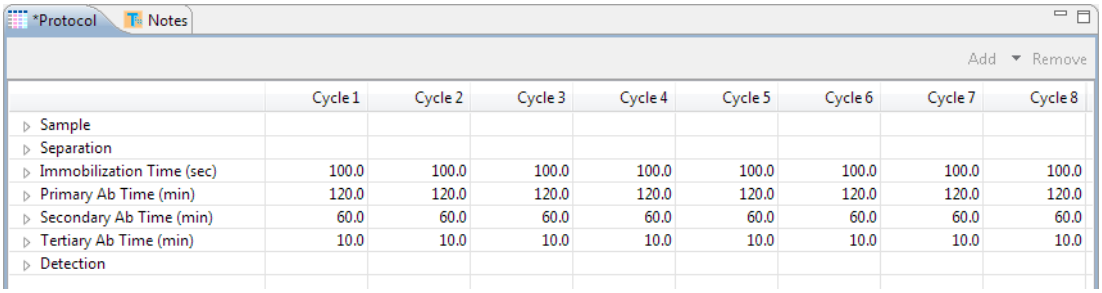
7. Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK.**



8. Modify any other protocol parameters as needed.
9. To add additional cycles to the assay protocol, click in any cell with a value in a cycle column.
   - To add one cycle, either click **Add** or click the down arrow next to Add and select **1 Cycle**
   - Select **4 Cycles** from the drop down menu to add four additional cycles
   - Select **All Cycles** from the drop down menu to add the number of cycles needed to reach the maximum of eight

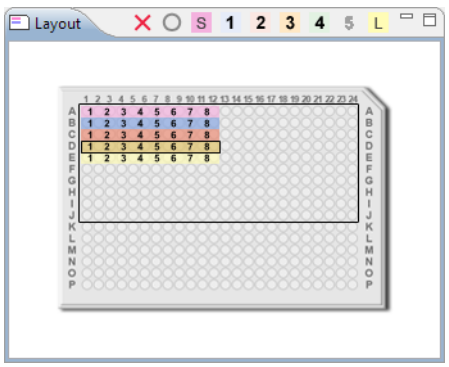


New cycle columns will display using the same parameters used for cycle 1.



	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Sample								
Separation								
Immobilization Time (sec)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Tertiary Ab Time (min)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Detection								

Repeat steps 1-8 to change parameters for the added cycles. As cycles are added and reagent locations are selected, cycle number assignments will update in the Layout pane assay plate map:

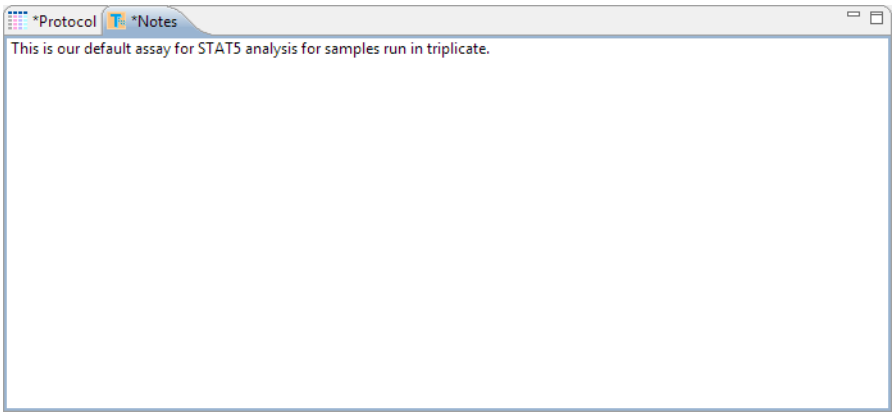


NOTES:
For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Step 4 - Add Assay Notes (Optional)

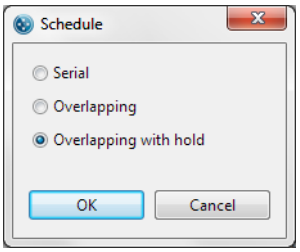
1. Click on the Notes tab.
2. Click in the pane and type any assay or protocol notes. This information will be stored with the assay and run data.



NOTE: When notes are being edited, an asterisk will appear in the Notes tab to indicate changes have been made and should be saved.

Step 5 - Select a Schedule (Optional)

Peggy can execute cycles serially or in parallel. To choose an option, select **Edit** and click **Schedule**.



- **Serial** - Each cycle is executed in sequential order, and each cycle completes before the next one starts. Selecting this option result in the longest run times.
- **Overlapping** - Cycle steps are executed in parallel if timing permits shared use of the separation tray and robot. Each cycle will run through to completion once started. This option offers a reduction in overall run time without affecting the individual run time for each cycle.
- **Overlapping with Hold (default)** - Each cycle executes through the separate step in sequence and then enters a variable length hold step where capillaries remain in the incubation tray. More sample stability is provided with this option as proteins are 'locked' to the capillary wall and therefore more stable than samples on the assay plate. This option also results in the most significant reduction in overall run time.

NOTE: ProteinSimple recommends using the overlapping with hold option. For more information on when to use other schedule options, please contact ProteinSimple Technical Support.

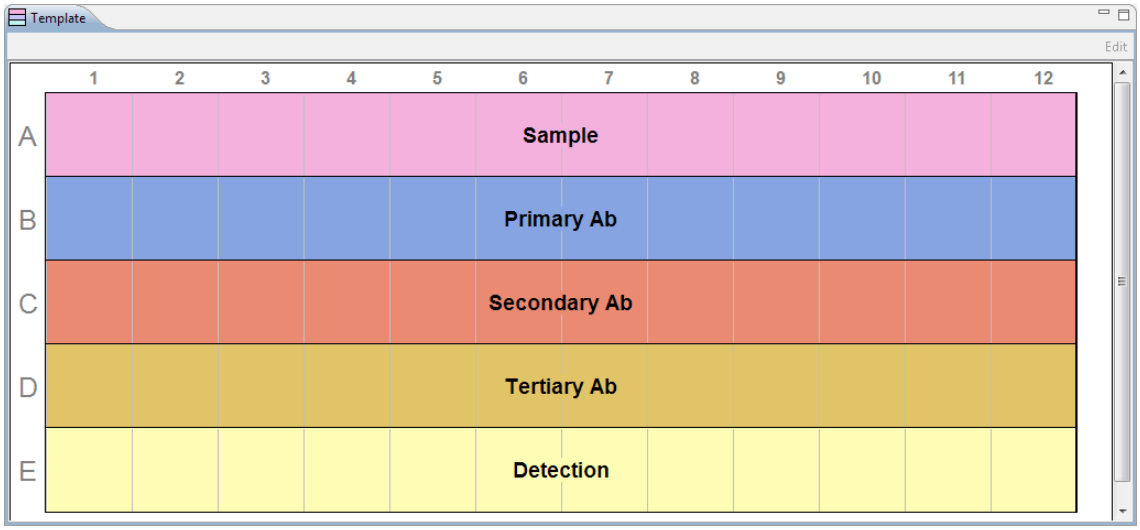
Step 6 - Add Assay Plate Annotations (Optional)

Custom reagent names, notes and annotations can be entered for individual rows and wells on the assay plate. This information will be stored with assay and run data. During post-run analysis, this information will be used to apply custom analysis settings to specific sample names, primary antibody names or sample and primary antibody attributes. This will be explained in more detail in "Compass Analysis Settings Overview" on page 219.

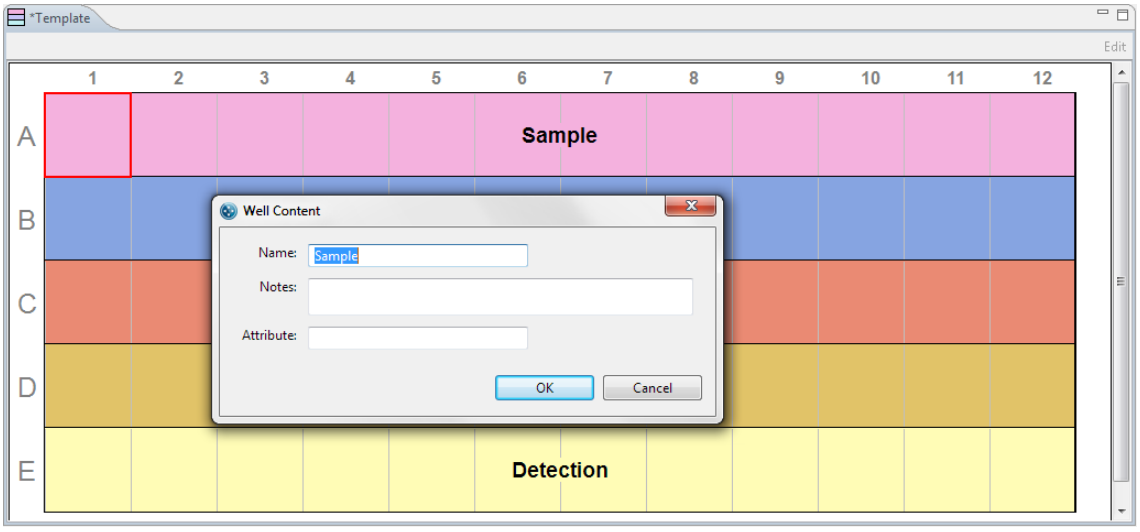
NOTE: Template pane information can also be added or updated after a run is complete.

To enter annotations:

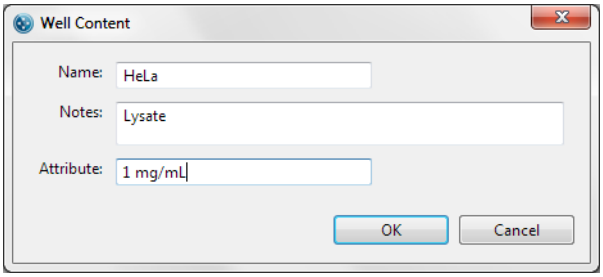
1. Click on the Template tab. The default annotations for reagent rows and individual wells on the assay plate will display:



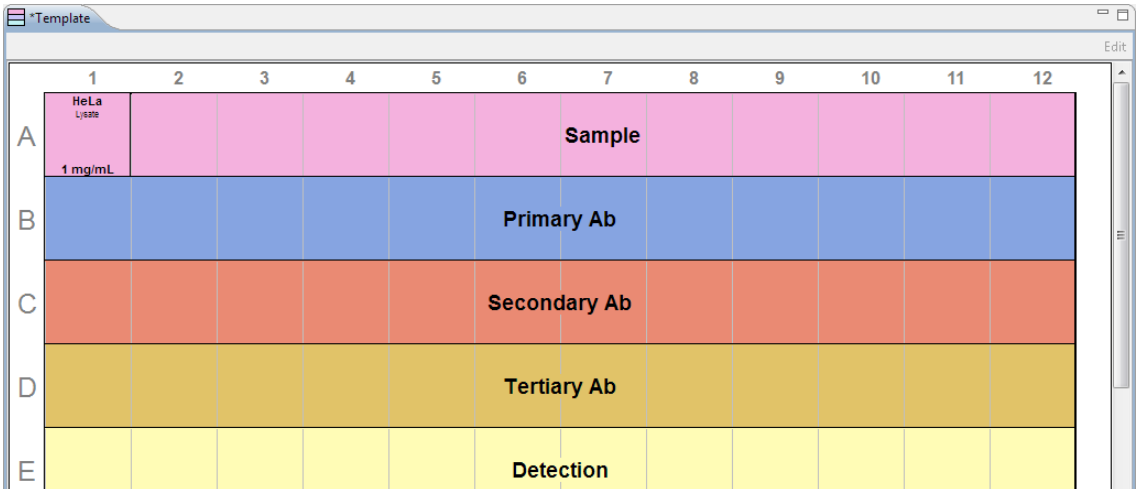
2. Change or add row and well annotations as needed. To do this:
   a. **To enter annotations for a specific well** - Right click the well and select **Edit** or click **Edit** in the upper right corner of the pane or double click the selected well. The following box will display:



Enter the reagent name, notes or specific attributes (for example, sample concentration or dilution ratio):

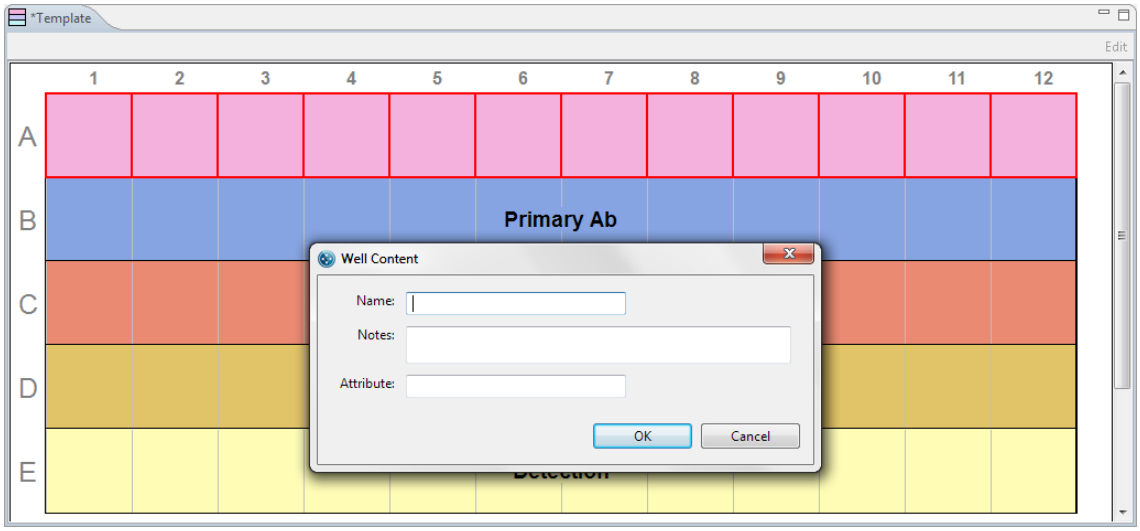


Click **OK**. The new information will display in the selected well:

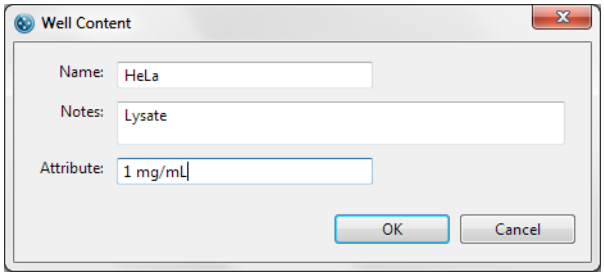


b. **To enter annotations for multiple wells or a row** - To select individual wells, press and hold the Control key and then select individual wells. To select a sequential set of wells or a full row, press and

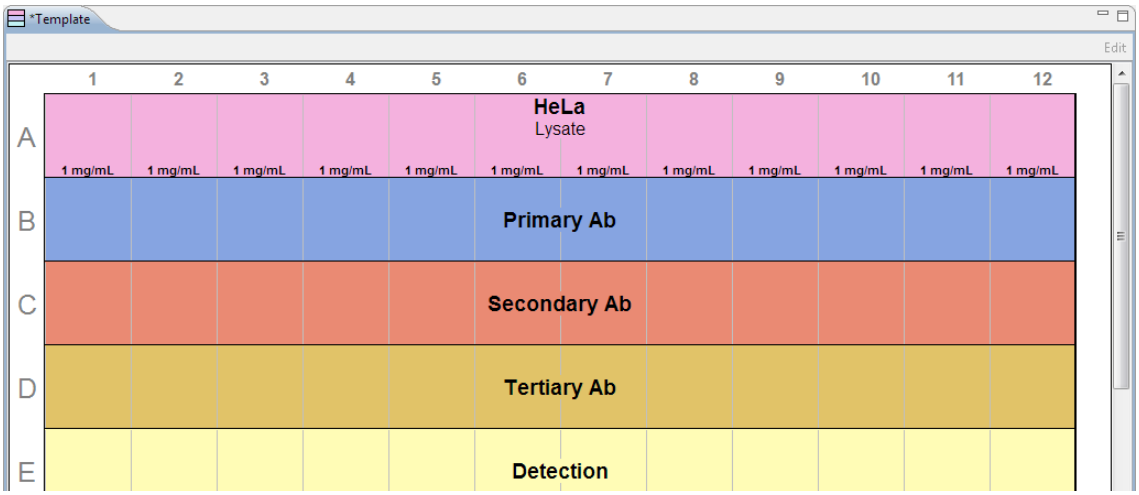
hold the Shift key, then select the first well and last well. Next, right click and select **Edit** or click **Edit** in the upper right corner of the pane. The following box will display:



Enter the reagent name, notes and specific attributes (for example, sample concentration or dilution ratio):



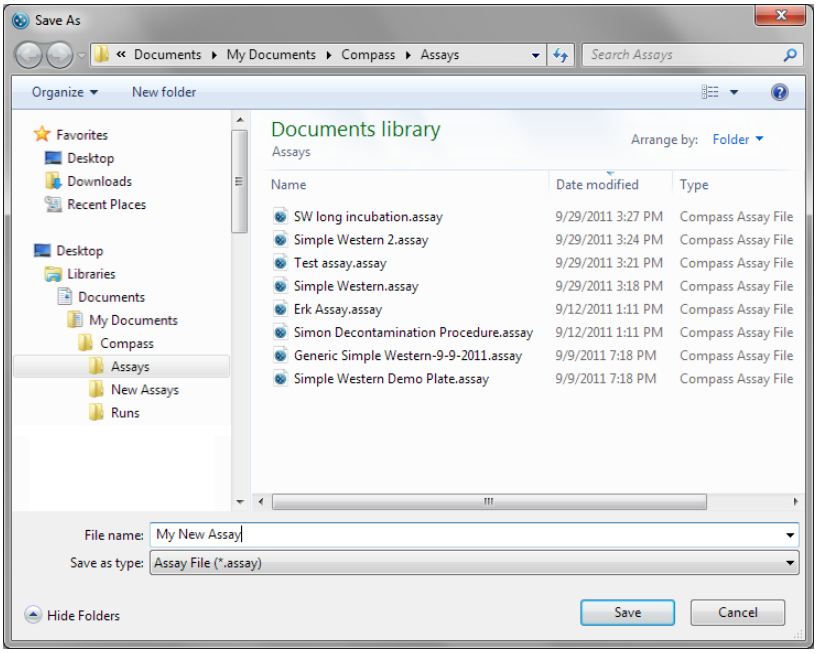
Click **OK**. The new information will display in the selected wells:



*NOTE: When annotations are being edited, an asterisk will appear in the **Template** tab to indicate changes have been made and should be saved. Only Name and Attribute will be used by Compass to annotate the data.*

Step 7 - Save the Assay

1. Select **File** from the main menu and click **Save As**. Enter the assay name and click **Save**.

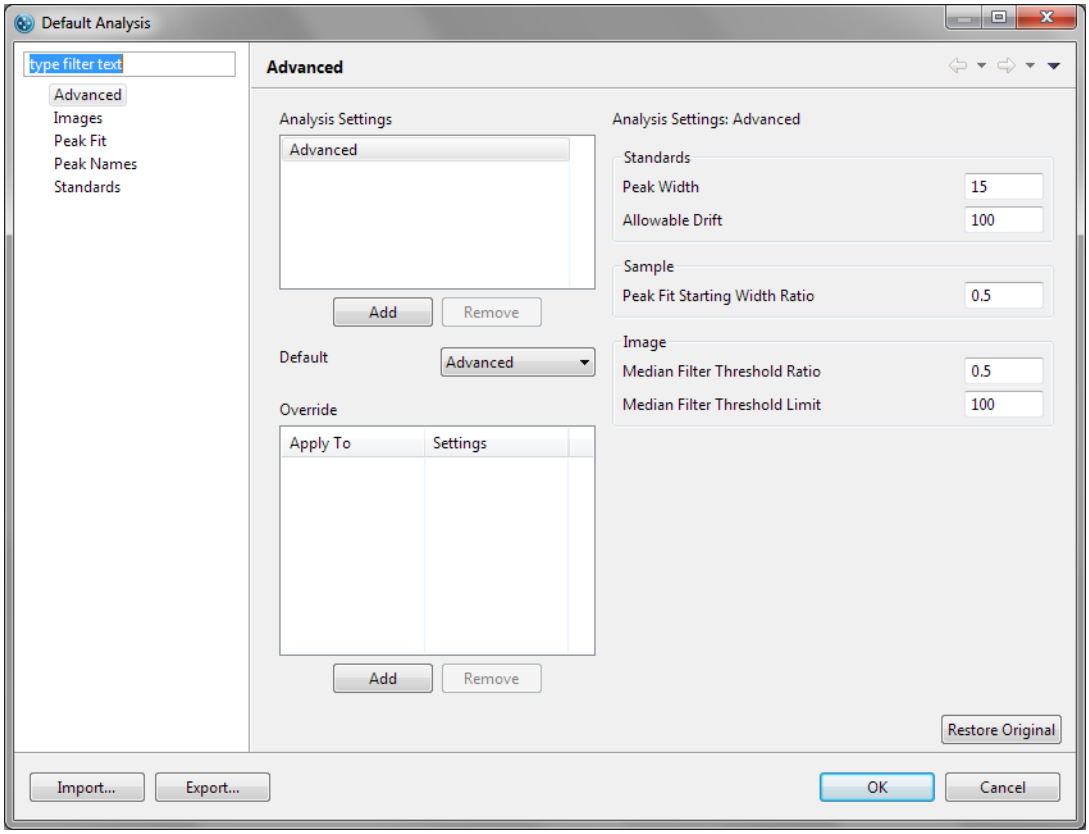


NOTE: New assays are saved in the Compass Assays directory.

Step 8 - Modify Default Analysis Parameters (Optional)

You can preset the parameters used to analyze run data generated with the assay.

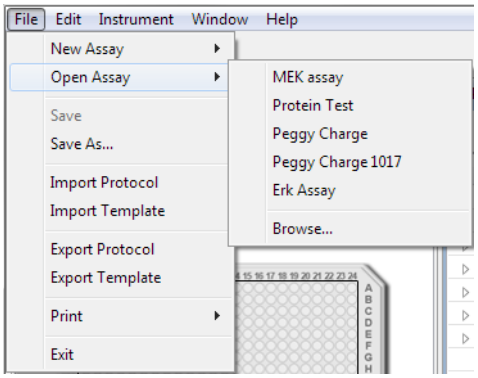
1. Select **Edit** from the main menu and click **Default Analysis**. The following screen will display:



2. ProteinSimple recommends using the default parameters for Simple Western assays. However, users can modify any parameters as needed, then click **OK**. For detailed information on analysis parameters, please refer to "Compass Analysis Settings Overview" on page 219.

Making Changes to an Existing Assay

1. Select **File** in the main menu and click **Open Assay**.

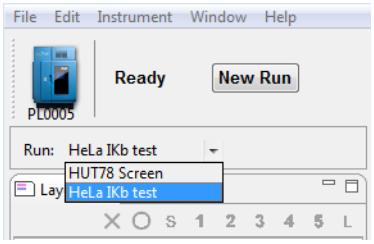


2. A list of the last assays opened will display. Select one of these assays or click **Browse** to open the assay folder and select a different assay.
3. Follow the steps in "Creating a New Assay" on page 67 to make changes and save the assay.

Switching Between Open Assays

If more than one run file is open, you can switch between viewing the assays for each run. To do this:

1. Click the down arrow in the run box.

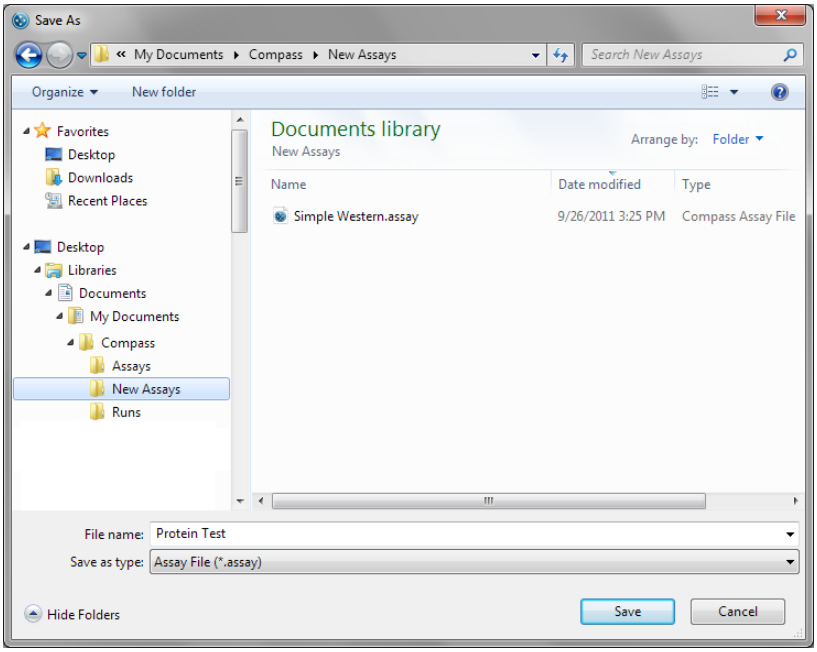


2. Select the run for the assay you want to view from the drop down list.

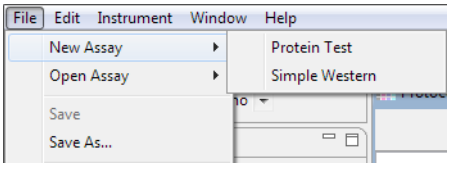
Creating a Template Assay

Users can create and save template assays that can be used as a starting point for creating new assays. To do this:

1. Select **File** in the main menu. Click **Open Assay** to open an existing assay or click **New Assay** to open an existing template assay.
2. Follow the steps in "Creating a New Assay" on page 67 to make changes to the assay.
3. When changes are complete, select **File** in the main menu and click **Save As**. Select the New Assays folder:

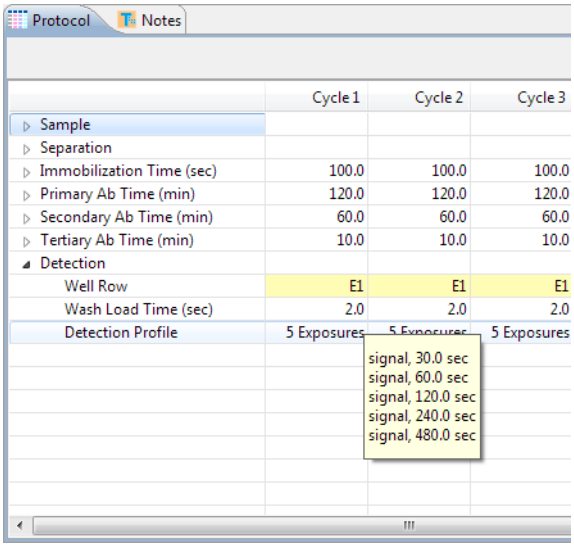


4. Type the name for the new template assay and click **Save**.
5. Select **File** in the main menu and click **New Assay**. The new template assay will now be available in the drop down list:



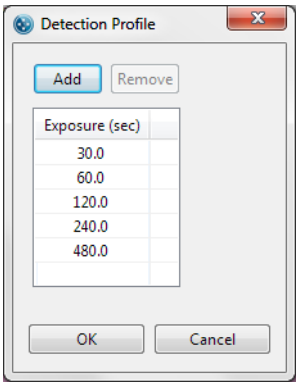
Viewing and Changing the Detection Exposures

To view the current detection profile, roll the cursor over the exposures cell in Protocol pane:



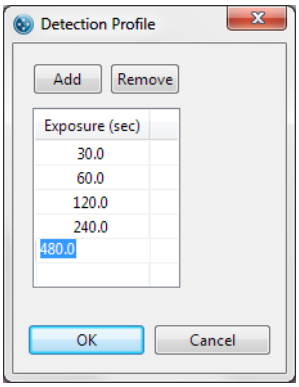
While ProteinSimple recommends using the default assay detection profile, users can change the profile, exposure times and exposure sequences if needed. To do this:

1. Select the exposures cell in the Protocol pane and click the button, or double-click in the cell. The following screen will display:



Each row represents an individual exposure that will be taken during the run.

a. **To change an existing exposure time** - Click in the exposure cell and enter a new time in seconds:



b. **To delete an existing exposure** - Select a type or exposure cell and click **Remove**.

c. **To add a new exposure** - Select **Add**. A new exposure will be added to the end of the list. Click in the exposure cell and enter an exposure time in seconds.

2. Click **OK** to save and exit.

Copying Protocols and Templates

The steps and parameters in the Protocol pane can be copied and d into other documents, as can the graphic image of the annotations in the Template pane.

Copying an Assay Protocol

1. Click on the Protocol tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Word, Excel, etc). Right click in the document and select. All assay protocol steps and parameters will be copied into the document as a list in the same format shown in the Protocol pane.

Copying an Assay Template

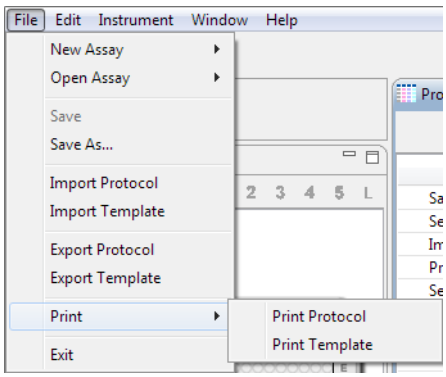
1. Click on the Template tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Word, Excel, etc). Right click in the document and select. The assay template will be copied into the document as an image, exactly as it is shown in the Template pane.

Printing Protocols and Templates

The information in the Protocol pane can be printed, as can a graphic image of the information in the Template pane.

Printing an Assay Protocol

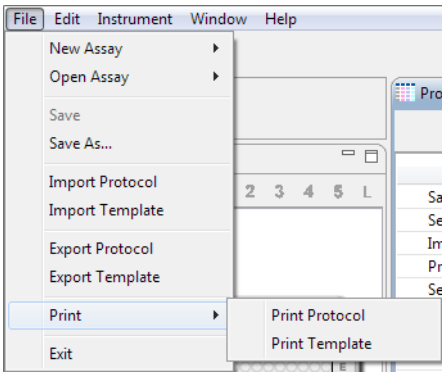
1. Click on the Protocol tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Protocol**.



All assay protocol steps and parameters will be printed as a list in the same format shown in the Protocol pane.

Printing an Assay Template

1. Click on the Template tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Template**.



The assay template will print as an image, exactly as it is shown in the Template pane.

Importing and Exporting Protocols and Templates

The assay protocol in an open assay or run file can be exported as a separate file, as can the assay template annotation information. This allows the same assay protocol and template information to be imported into another assay at a later time, rather than having to re-enter assay protocol and template information manually.

Importing an Assay Protocol

NOTE: Importing an assay protocol imports information into the Protocol pane only.

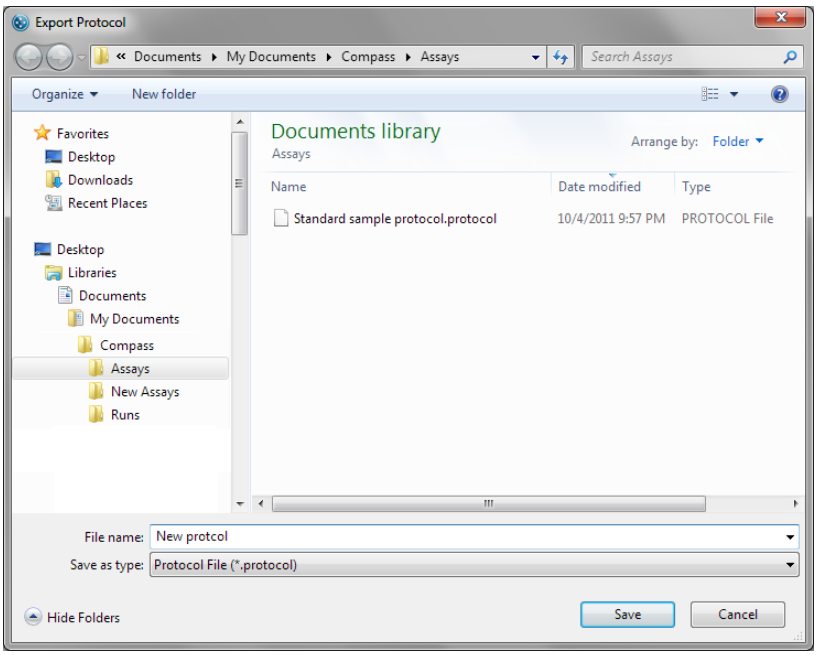
---

1. Open the assay you want to import the assay protocol in to.
2. Select **File** in the main menu and click **Import Protocol**.
3. Select a protocol file (*.protocol) and click **OK**. The imported information will display in the Protocol pane.

Exporting an Assay Protocol

NOTE: Exporting an assay protocol exports information in the Protocol pane only.

1. Open the assay you want to export the assay protocol from.
2. Select **File** in the main menu and click **Export Protocol**. The following window displays:



3. The default directory is Compass/Assays. Change the directory if needed.
4. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file.

Importing an Assay Template

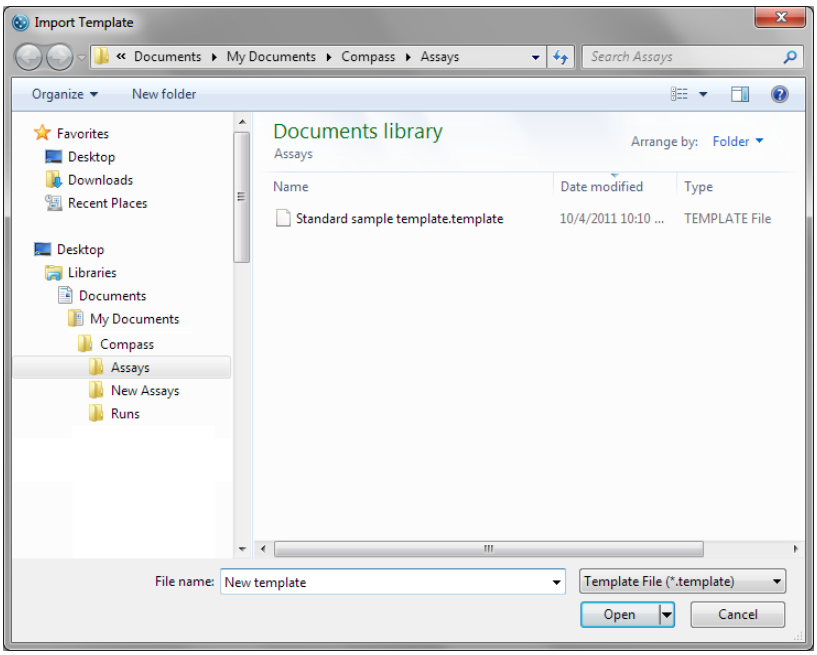
NOTE: Importing an assay template imports information into the Template pane only.

1. Open the assay you want to import the assay template in to.
2. Select **File** in the main menu and click **Import Template**.
3. Select a template file (*.template) and click **OK**. The imported information will display in the Template pane.

Exporting an Assay Template

NOTE: Exporting an assay template exports information in the Template pane only.

1. Open the assay you want to export the assay template from.
2. Select **File** in the main menu and click **Export Template**. The following window displays:



3. The default directory will be Compass/Assays. Change the directory if needed.
4. Enter a template name and click **Save**. The template will be saved as a *.template file.

Chapter 5:

Running a Charge Assay on Peggy

Chapter Overview

- Starting a Run
- Stopping a Run

Starting a Run

Step 1 - Get Ready

1. Open Compass software.
2. Prepare instrument: empty waste, refill water and add a new manifold sponge.
3. Create or open desired assay file.
4. Prepare assay plate following the procedure described in the product insert.

IMPORTANT
To prevent well evaporation and ensure best results, keep a lid on the assay plate until ready to use.

---

5. While plate is spinning, add Wash Buffer, Anolyte and Catholyte to resource tray cups. Place capillary box in the designated resource tray position.

IMPORTANT
Capillaries are light sensitive. Keep the cover on the box until you are ready to transfer the capillary box to the resource tray.

---

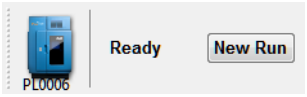
6. Place assay plate into the sample tray of the instrument and press **Start**.

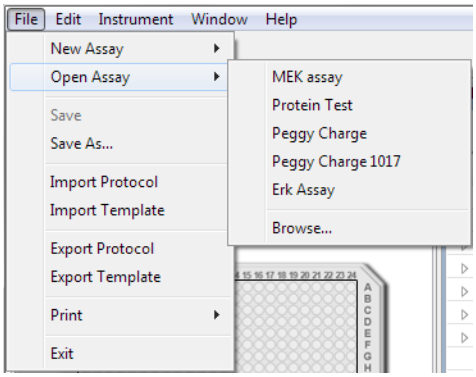
Step 2 - Start the Run

1. You can start a run in one of two ways depending on what button is displayed in the status bar.

   NEW RUN BUTTON- click **New Run**.

a.Select **File** in the main menu and click **Open Assay**.



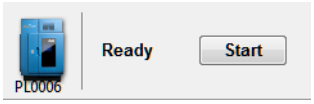


b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

c. Alternatively, choose **New Assay** and select **Peggy Charge** to get the default Peggy assay conditions.

d. The **Start** button will display.

START BUTTON - this indicates an assay is already loaded.



a. Go to the Assay screen and verify this is the assay you want to use. If not, select **File** in the main menu, click **Open Assay**, and select another assay.

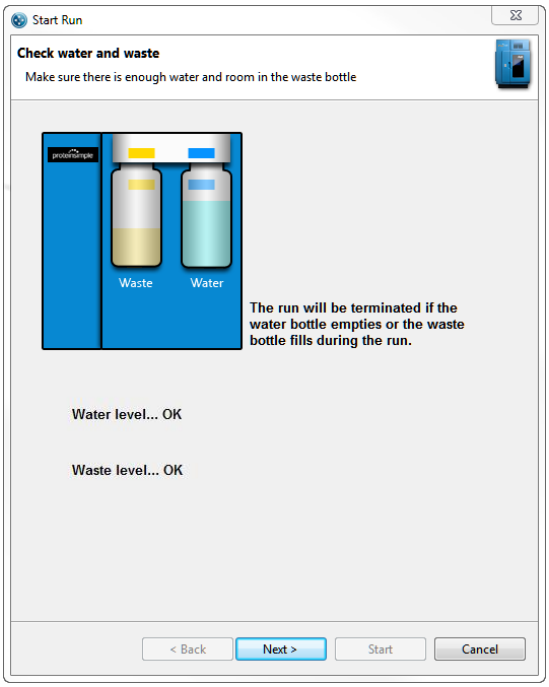
2. Click **Start**. This will launch the **Start Run Wizard**.

---

*NOTE: If the manifold was not cleaned prior to starting the run, a message indicating this will display. If this occurs, click **Yes** to cancel the run and perform the manifold cleaning.*

---

3. **Page 1: Check Water and Waste.** The fluid levels in the accessory module bottles will be checked by the software. If the levels in both bottles will allow Peggy to complete the run, the wizard screen will display **Water Level OK** and **Waste Level OK** messages. Click **Next** to proceed.

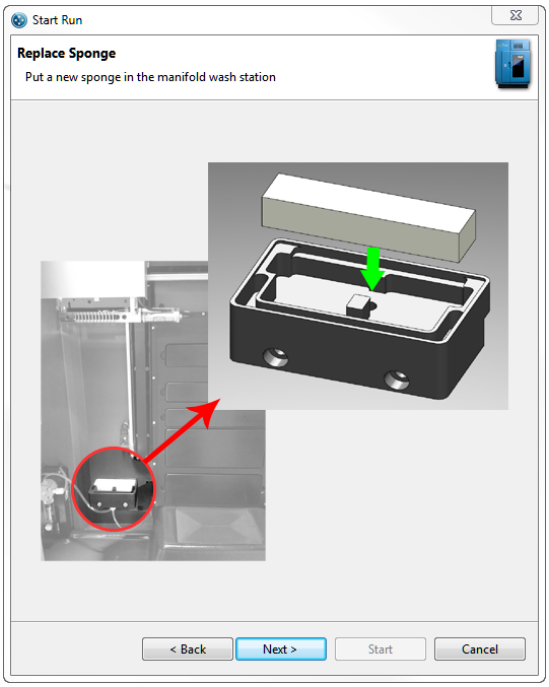

Start Run
Check water and waste
Make sure there is enough water and room in the waste bottle
Waste
Water
The run will be terminated if the water bottle empties or the waste bottle fills during the run.
Water level... OK
Waste level... OK
< Back
Next >
Start
Cancel

---

*NOTE: If the waste level is too high or the water level is too low to complete the run, messages to indicate one or both will be presented in this screen. If this occurs, fill or empty each bottle as indicated using the procedures outlined in the Peggy User Guide. When this is complete, the error status will be automatically updated and allow the **Start Run Wizard** to proceed.*

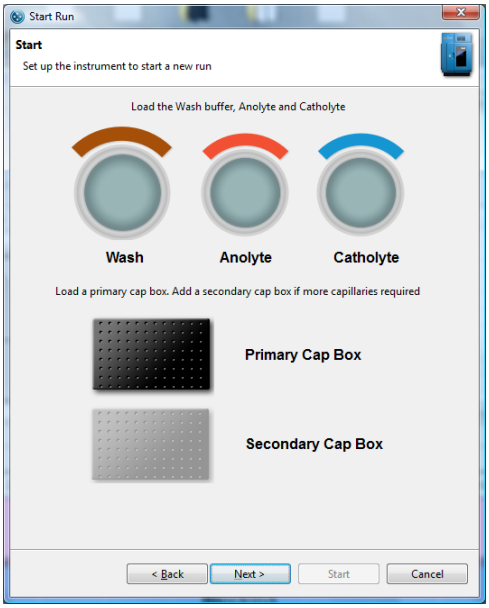
---

4. **Page 2: Replace Sponge.** A new sponge should be used each time a new experimental run is started. Discard the old sponge and put a new sponge in the manifold wash station.



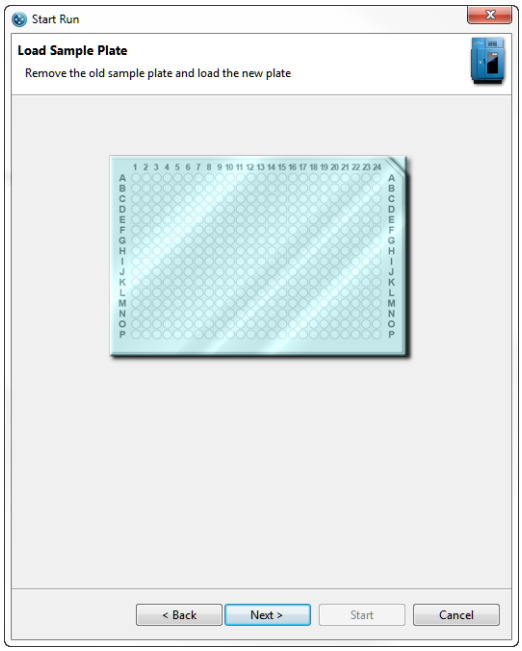
5. **Page 3: Start.** The resource tray will automatically open. Fill the Wash Buffer, Anolyte and Catholyte cups and insert the capillary box in the primary capillary box position. Add a secondary capillary box if more capillaries are required. Click **Next** when finished. The resource tray will close.

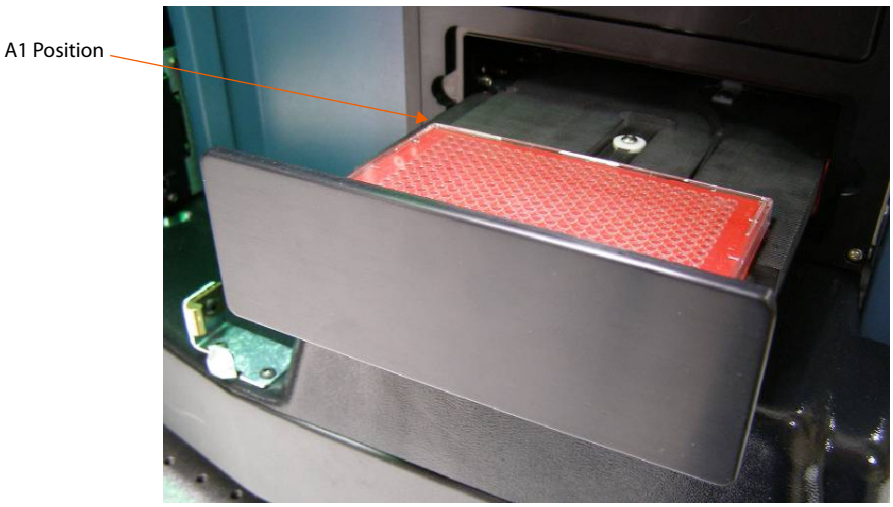
NOTE: Make sure enough capillaries are loaded to complete the run. A capillary box should be inserted in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is depleted, Peggy will automatically move to the secondary box. Discard leftover Running Buffer, Wash Buffer and Matrix Removal Buffer prior to refilling bottles for new run.



NOTE: You an also refer to the labels on the resource tray for proper insertion of reagents.

6. **Page 4: Load Sample Plate.** The sample tray will automatically open. Place the lidded 384-well plate on the cooling block in the tray, ensuring that the A1 well position is aligned with the upper left corner of the cold block. When this is complete, click **Next**. The sample tray will close.





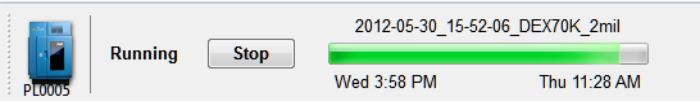
NOTES:
Peggy requires that plate lids be used on sample plates. If a lid is not detected, a message will be displayed in the Start Run Wizard. Compass software will reopen the sample tray to allow the user to insert a lid.

When inserting the sample plate, ensure that the plate is firmly seated and level on the cold block. Plates that are not level can interfere with the movement of the sample tray.

---

7. **Page 5: Data File.** The data file name will automatically default to the assay name appended with the current date and time. To change the file name, begin typing in the text box. To change the directory where the data file will be stored, click **Browse**:

Click **Start** to begin the run. Instrument status will change to running, and the stop button and progress bar will display:



The run will continue until complete (~14-19 hours depending on the assay).

Step 3 - Post-Run Procedures

1. Empty the capillary discard tray.
2. Remove the assay plate.
3. Dispose of the assay plate and capillaries. Disposal will depend on the samples that have been assayed. If sample origins are unknown, ProteinSimple recommends that used capillaries and plates be disposed of in biohazard waste.

!WARNING! SHARPS HAZARD

The capillaries may present a potential sharps hazard. Dispose of used capillaries in biomedical waste sharps containers.

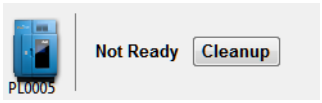
!WARNING! BIOHAZARD

Samples and waste bottle contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations. Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste vial before you store, handle, or dispose of chemical waste.

Stopping a Run

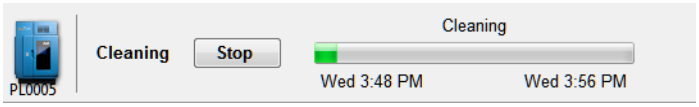
1. To stop a run, click **Stop**. When the run stops, instrument status will go to Not Ready and a Cleanup button displays:



NOTE: If a run is stopped prior to completion, Peggy must perform a cleaning protocol to remove used capillaries from system trays. This is required to prepare the system for the next run.

---

2. Click **Cleanup**.



Allow Peggy to complete the cleaning protocol which takes about ten minutes. When complete, instrument status will change to Ready and a new run can be started.

Chapter 6:

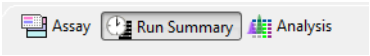
Run Status

Chapter Overview

- Run Summary Screen Overview
- Opening Run Files
- Viewing File and Run Status Information
- Watching Standards Separation Movies
- Viewing Current and Voltage Plots
- Switching Between Open Run Files
- Closing Run Files

Run Summary Screen Overview

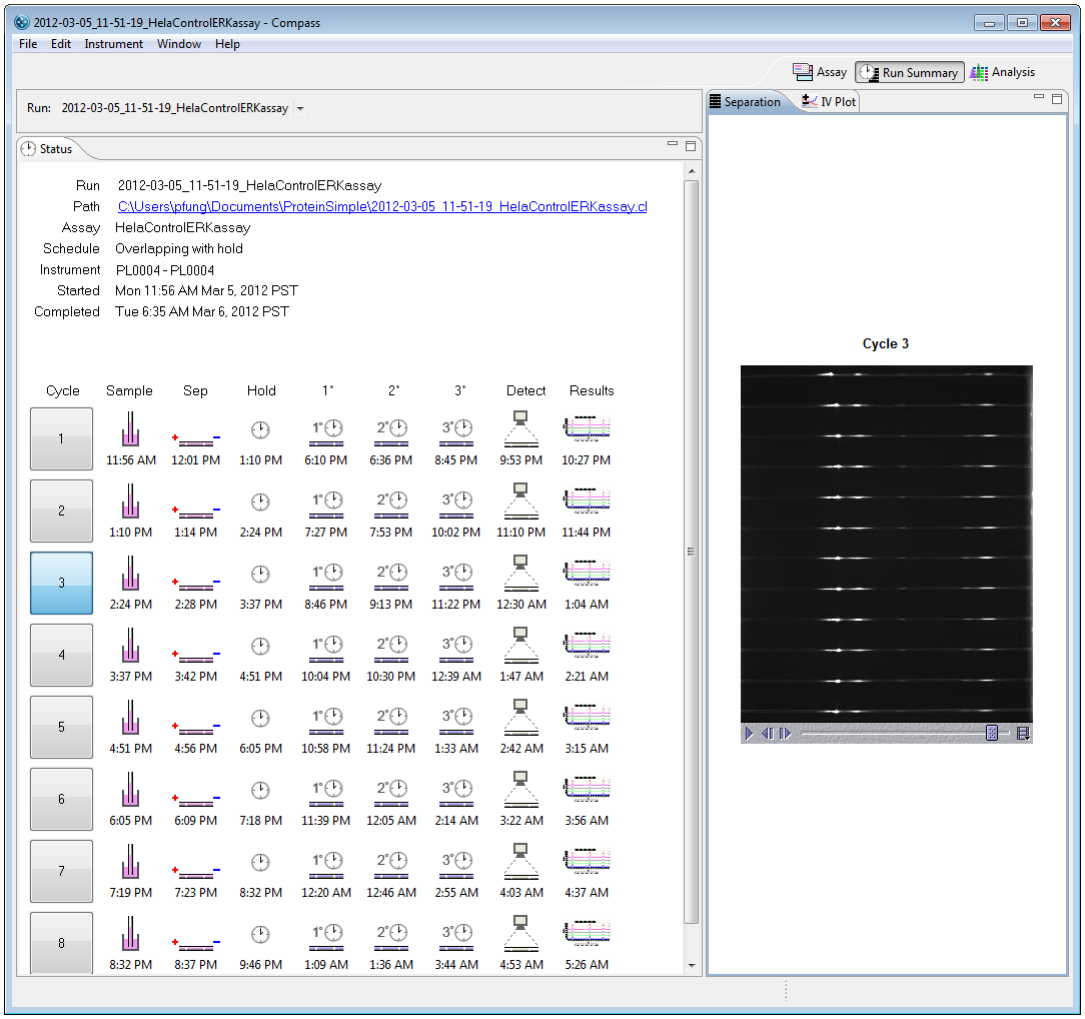
The Run Summary screen is used to monitor run progress, watch movies of the fluorescent standards separation, and view current and voltage plots for a run. To access this screen, click Run Summary in the screen tab:



Run Summary Screen Panes

The Run Summary screen has three panes:

- **Status** - Displays run file information and current status of a run in progress.
- **Separation** - Lets you view a movie of the fluorescent standards separation for each cycle of the experimental run.
- **IV Plot** - Lets you view plots of the total current and voltage measured during separation for all 12 capillaries for each cycle of the experimental run.


2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit Instrument Window Help
Assay Run Summary Analysis
Run: 2012-03-05_11-51-19_HelaControlERKassay
Separation IV Plot
Status
Run 2012-03-05_11-51-19_HelaControlERKassay
Path C:\Users\pfung\Documents\ProteinSimple\2012-03-05_11-51-19_HelaControlERKassay.cl
Assay HelaControlERKassay
Schedule Overlapping with hold
Instrument PL0004 - PL0004
Started Mon 11:56 AM Mar 5, 2012 PST
Completed Tue 6:35 AM Mar 6, 2012 PST
Cycle 3
Cycle Sample Sep Hold 1° 2° 3° Detect Results
1 11:56 AM 12:01 PM 1:10 PM 6:10 PM 6:36 PM 8:45 PM 9:53 PM 10:27 PM
2 1:10 PM 1:14 PM 2:24 PM 7:27 PM 7:53 PM 10:02 PM 11:10 PM 11:44 PM
3 2:24 PM 2:28 PM 3:37 PM 8:46 PM 9:13 PM 11:22 PM 12:30 AM 1:04 AM
4 3:37 PM 3:42 PM 4:51 PM 10:04 PM 10:30 PM 12:39 AM 1:47 AM 2:21 AM
5 4:51 PM 4:56 PM 6:05 PM 10:58 PM 11:24 PM 1:33 AM 2:42 AM 3:15 AM
6 6:05 PM 6:09 PM 7:18 PM 11:39 PM 12:05 AM 2:14 AM 3:22 AM 3:56 AM
7 7:19 PM 7:23 PM 8:32 PM 12:20 AM 12:46 AM 2:55 AM 4:03 AM 4:37 AM
8 8:32 PM 8:37 PM 9:46 PM 1:09 AM 1:36 AM 3:44 AM 4:53 AM 5:26 AM

Software Menus Active in the Run Summary Screen

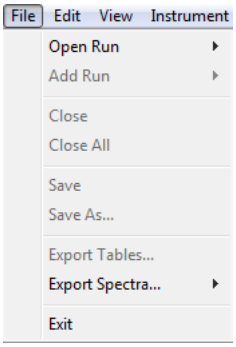
The following software menus are available:

- File
- Edit
- Instrument (when Compass is connected to Peggy)
- Window
- Help

The File and Edit menu options specific to the Run Summary screen are described next.

File Menu

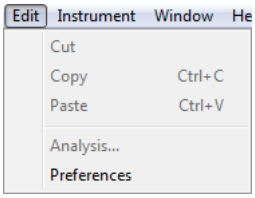
The following **File** menu options are active:



- **Open Run** - Opens a run file.
- **Add Run** - Open and view other run files in addition to the one that is already open.
- **Close** - Closes the run file currently being viewed.
- **Close All** - Closes all open run files.
- **Exit** - Closes Compass.

Edit Menu

The following Edit menu options are active:



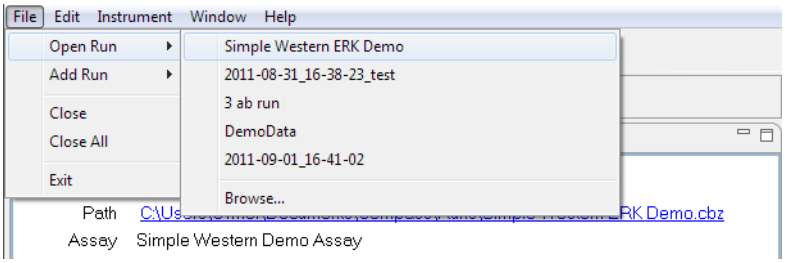
- **Preferences** - Set and save custom preferences for data export, plot colors in the graph and Peggy's Twitter settings. See Chapter 10, *"Setting Custom User Preferences"* for more information.

Opening Run Files

You can open one run file or multiple run files at a time to compare information between runs.

Opening One Run File

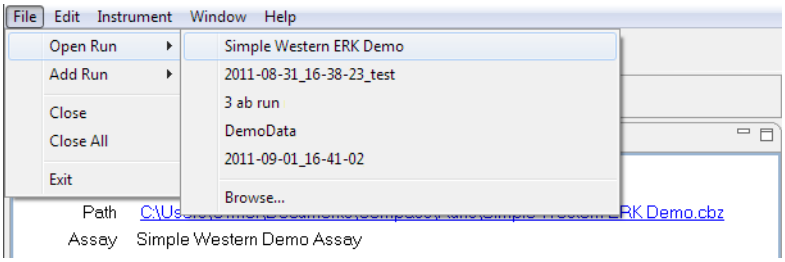
1. Select **File** in the main menu and click **Open Run**.



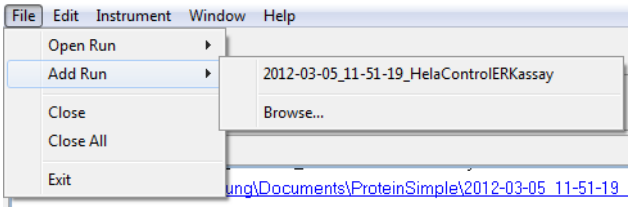
2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

Opening Multiple Run Files

1. To open the first run file, select **File** in the main menu and click **Open Run**.



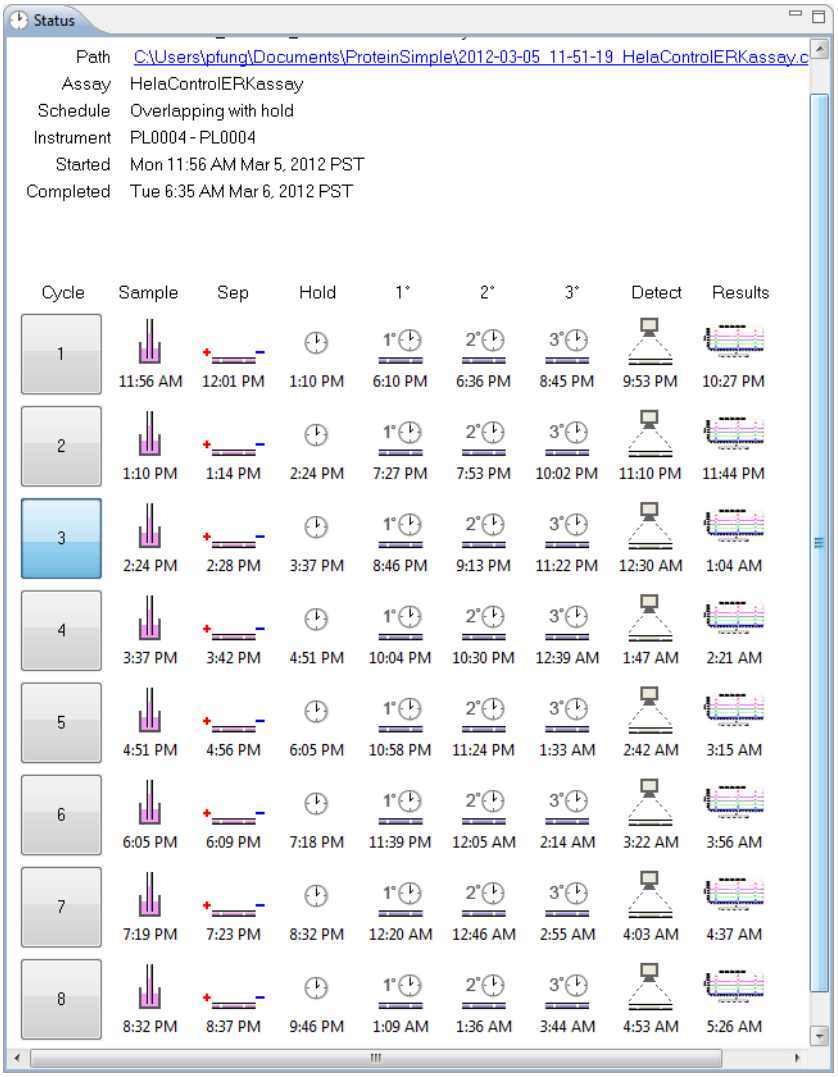
2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
3. To open another run file, select **File** in the main menu and click **Add Run**.



4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
5. Repeat the last two steps to open additional runs.

Viewing File and Run Status Information

Information specific to each run file is shown in the Status pane:


Status
Path C:\Users\pfung\Documents\ProteinSimple\2012-03-05_11-51-19_HelaControlERKassay.c
Assay HelaControlERKassay
Schedule Overlapping with hold
Instrument PL0004 - PL0004
Started Mon 11:56 AM Mar 5, 2012 PST
Completed Tue 6:35 AM Mar 6, 2012 PST
Cycle Sample Sep Hold 1° 2° 3° Detect Results
1 11:56 AM 12:01 PM 1:10 PM 6:10 PM 6:36 PM 8:45 PM 9:53 PM 10:27 PM
2 1:10 PM 1:14 PM 2:24 PM 7:27 PM 7:53 PM 10:02 PM 11:10 PM 11:44 PM
3 2:24 PM 2:28 PM 3:37 PM 8:46 PM 9:13 PM 11:22 PM 12:30 AM 1:04 AM
4 3:37 PM 3:42 PM 4:51 PM 10:04 PM 10:30 PM 12:39 AM 1:47 AM 2:21 AM
5 4:51 PM 4:56 PM 6:05 PM 10:58 PM 11:24 PM 1:33 AM 2:42 AM 3:15 AM
6 6:05 PM 6:09 PM 7:18 PM 11:39 PM 12:05 AM 2:14 AM 3:22 AM 3:56 AM
7 7:19 PM 7:23 PM 8:32 PM 12:20 AM 12:46 AM 2:55 AM 4:03 AM 4:37 AM
8 8:32 PM 8:37 PM 9:46 PM 1:09 AM 1:36 AM 3:44 AM 4:53 AM 5:26 AM

The run file name, path (directory location) and assay used is displayed along with instrument serial number and the run start/complete date and time.

- **To go to the run file directory location** - Double click the path hyperlink, or right-click and select **Open Directory**.

- **To copy the path** - Right-click on the path hyperlink and click **Copy**. The path can then be copied into documents. The path can also be copied into the Windows Explorer address bar to launch Compass and open the run file automatically.

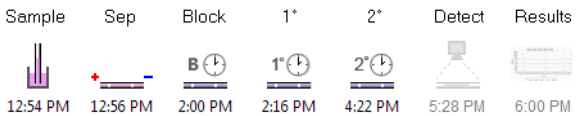
Assay Steps: Size-based Assays

Each assay step that is executed during the run is represented by an icon. The time the individual step is scheduled to start is shown under each icon. Details on what happens during each assay step is as follows:

Step	Description
	Sample Loading Step - Capillaries are moved to the assay plate in the sample tray where Separation Matrix, Stacking Matrix, biotinylated ladder and samples are aspirated. Capillaries are then transferred to the separation tray.
	Separation Step - Samples and fluorescent standards are separated in the capillaries. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. During the separation, voltage and current are monitored and displayed in the IV Plot. Separation of the fluorescent standards is recorded and a movie of the separation is compiled for viewing when separation is complete in the Separation pane. After separation, Matrix Removal Buffer is aspirated.
	Blocking Step - Capillaries are moved to the assay plate in the sample tray and blocking reagent (Antibody Diluent) is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
	Primary Antibody (1°) Step - Capillaries are moved to the assay plate in the sample tray and primary antibody is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
	Secondary Antibody (2°) Step - Capillaries are moved to the assay plate in the sample tray and secondary HRP conjugate and Streptavidin-HRP is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
	Detect Step - Capillaries are moved to the assay plate in the sample tray and Luminol-Peroxide solution is aspirated. Capillaries are then transferred to the separation tray where the emitted chemiluminescent light is detected with the CCD camera.

Step	Description
Results 6:00 PM	**Results Step -** Results are available in the Analysis screen.
Hold 1:10 PM	**Hold Step -** This step is used for overlapping or overlapping with hold schedule options. A cycle that has completed a specific step will go into a hold step while the remaining cycles execute the same step in parallel. For example, once cycle1 completes the separate step, it may go into a hold step while cycle 2 executes the separate step. This execute and hold pattern will continue until all cycles have completed the separate step.

When a run is in progress, icons for steps that have not executed will be grey (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:



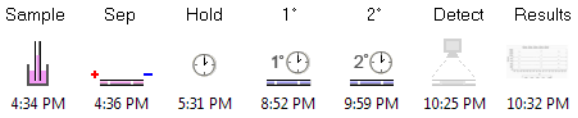
Assay Steps: Charge-based Assays

Each assay step that is executed during the run is represented by an icon. The time the individual step is scheduled to start is shown under each icon. Details on what happens during each assay step is as follows:

Step	Description
Sample 12:54 PM	**Sample Loading Step** - Capillaries are moved to the assay plate in the sample tray and samples are aspirated. Capillaries are then transferred to the separation tray.
Sep 12:56 PM	**Separation Step** - Samples, ampholyte mix and fluorescent pI standards are separated in the capillaries. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. During the separation, voltage and current are monitored and displayed in the IV Plot. Separation of the fluorescent standards is recorded and a movie of the separation is compiled for viewing in the Separation pane after separation is complete.

Step	Description
1° 2:16 PM	**Primary Antibody (1°) Step** - Capillaries are moved to the assay plate in the sample tray and primary antibody is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
2° 4:22 PM	**Secondary Antibody (2°) Step -** Capillaries are moved to the assay plate in the sample tray and secondary HRP-conjugated antibody is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
Detect 5:28 PM	**Detect Step -** Capillaries are moved to the assay plate in the sample tray and Luminol-Peroxide solution is aspirated. Capillaries are then transferred to the separation tray where the emitted chemiluminescent light is detected with the CCD camera.
Results 6:00 PM	**Results Step -** Results are available in the Analysis screen.
Hold 1:10 PM	**Hold Step -** This step is used for overlapping or overlapping with hold schedule options. A cycle that has completed a specific step will go into a hold step while the remaining cycles execute the same step in parallel. For example, once cycle1 completes the separate step, it may go into a hold step while cycle 2 executes the separate step. This execute and hold pattern will continue until all cycles have completed the separate step.

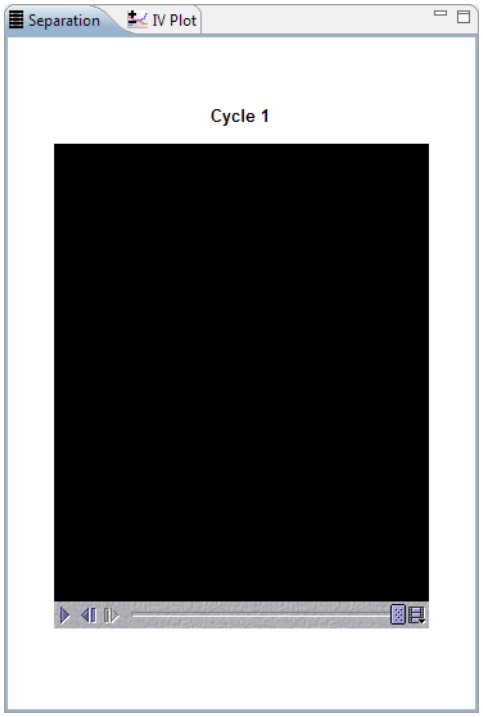
When a run is in progress, icons for steps that have not executed will be grey (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:



Watching Standards Separation Movies

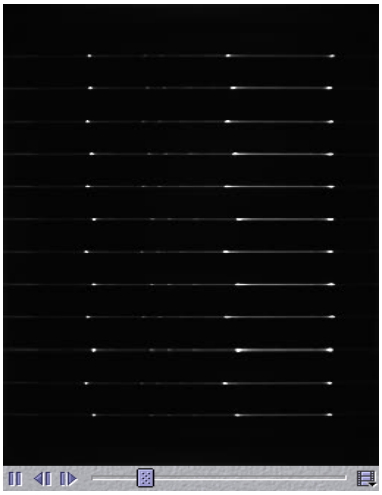
Users can view a movie of the fluorescent standards separation in all 12 capillaries. To do this:

1. Click the **Separation** tab.



2. The player control panel has play/pause, rewind and fast forward buttons, and a slider bar that allows you to scroll through the movie manually:

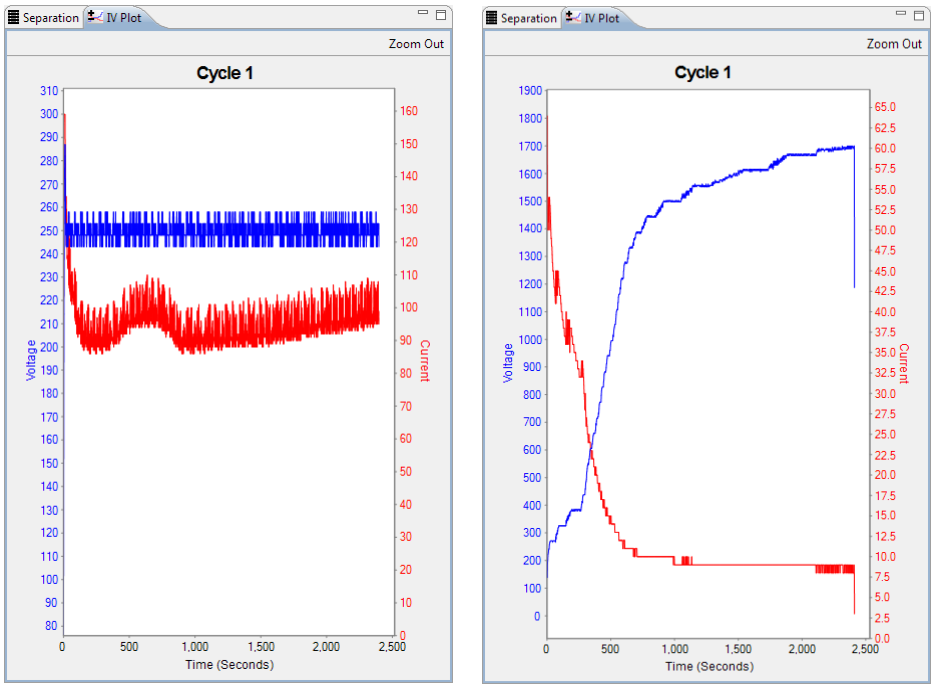
Click **Play** (button on far left) to view the movie. In the examples below, standards for a size assay are on the left, and standards for a charge assay are on the right:



NOTE: Complete separation movies of the fluorescent standards are not available until the separation step has finished executing. If the movie is played while the separation step is executing, the movie will only show separation progress up to the current point in time.

Viewing Current and Voltage Plots

Users can view plots of the total current and voltage measured during separation for all 12 capillaries. To do this, click the **IV Plot** tab. In the examples below, the IV plot for a size assay is on the left, and the IV plot for a charge assay is on the right:



The blue Y-axis and plot shows the run voltage in volts (V), and the red Y-axis and plot shows the run current in micro amps (µA). The X-axis displays time in seconds.

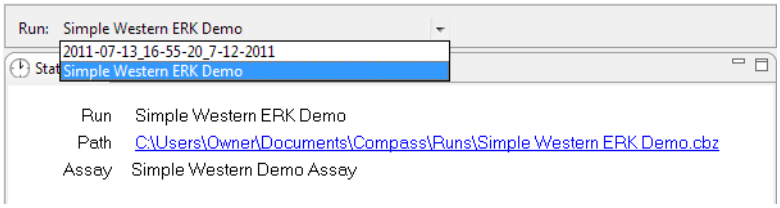
- **To zoom in on an area of the plot** - Hold the mouse button down and draw a box around the area with the mouse.
- **To zoom out** - Click **Zoom Out** in the upper right corner of the pane.

NOTE: The IV plot for a run in progress will not be available until the separation step starts executing. The plot is then displayed in real-time.

Switching Between Open Run Files

If more than one run file is open, you can switch between viewing the run information in each. To do this:

1. Click the down arrow in the run box.


Run: Simple Western ERK Demo
2011-07-13_16-55-20_7-12-2011
Simple Western ERK Demo
Run Simple Western ERK Demo
Path C:\Users\Owner\Documents\Compass\Runs\Simple Western ERK Demo.cbz
Assay Simple Western Demo Assay

2. Select the run you want to view from the drop down list.

Closing Run Files

If more than one run file is open, you can close just one file or all the open files at the same time.

- **To close the run file being viewed** - Select **File** from the main menu and click **Close**.
- **To close all open run files** - Select **File** from the main menu and click **Close All**.

Chapter 7:

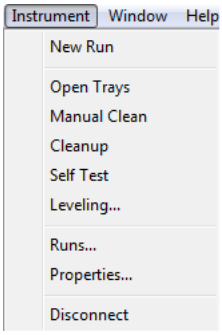
Controlling Peggy

Chapter Overview

- Instrument Control
- Self Test
- Viewing and Changing System Properties
- Viewing Log Files
- Peggy's Status Modes

Instrument Control

The Instrument menu allows users to control Peggy.



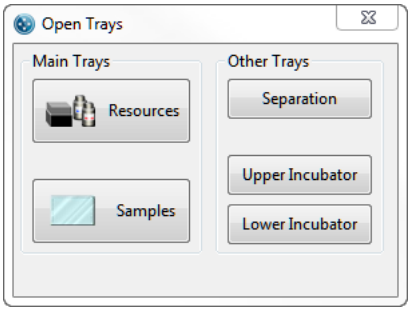
NOTE: Instrument menu options are active only when a computer with Compass software is connected directly to Peggy.

Starting a New Run

To start a new run, select **Instrument** in the main menu and click **New Run**. Then follow the steps described in "Step 2 - Start the Run" on page 52 for size assays or "Step 2 - Start the Run" on page 96 for charge assays.

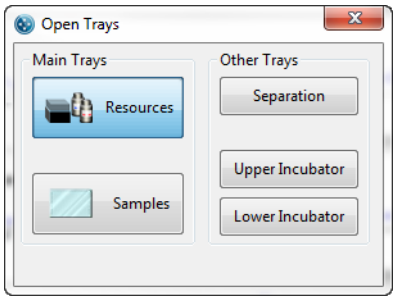
Opening Trays

To open any of the five trays, select **Instrument** and click **Open Trays**. The tray control window will appear:



Open a tray by clicking on its button. The button will become highlighted indicating the tray is open.

NOTE: Only one tray can be open at a time.



To close a tray, click the corresponding tray button again.

NOTE: If the tray control window is closed when a tray is open, the tray will close automatically.

Cleaning

Two cleaning options are available for Peggy.

Manual Clean

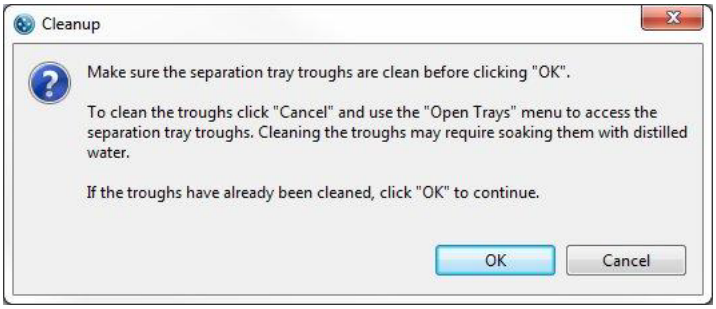
This option is used for general manual cleaning and cleaning the manifold head. To do a manual cleaning, select **Instrument** and click **Manual Clean**. The manifold head will move to a safe position for easy access and the vacuum will turn on.

NOTE: Please contact Protein Simple Technical Support if you have any questions regarding the manifold cleaning procedure.

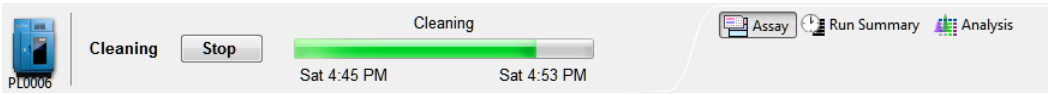
Cleanup

This option is a fully automated cleaning step. The manifold head is flushed, the separation tray troughs are aspirated and washed, and any capillaries left in the trays or gripper are picked up and discarded. This option should be selected when the instrument has not been used for more than a week or if a run error occurs. Cleaning takes about eight minutes to complete.

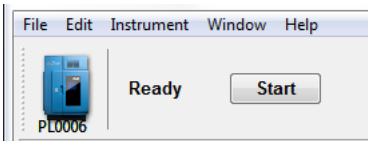
To start the protocol, select **Instrument** and click **Cleanup**. A window will appear with instructions:


Cleanup
Make sure the separation tray troughs are clean before clicking "OK".
To clean the troughs click "Cancel" and use the "Open Trays" menu to access the separation tray troughs. Cleaning the troughs may require soaking them with distilled water.
If the troughs have already been cleaned, click "OK" to continue.
OK
Cancel

Peggy's status will change to cleaning, and the stop button and the cleaning progress bar display. The **Assay** screen provides cleaning status details:



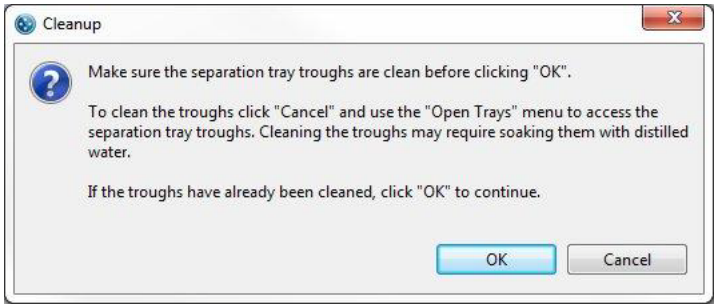
When cleaning is complete, instrument status will change to **Ready**.



Cleaning After a Run Error

Additional cleaning steps are required if an error occurs that stops the run. When this happens, the red Error status light on Peggy's front panel will come on.

Click on the **Reset** button displayed in Compass software. The following instructions will appear:

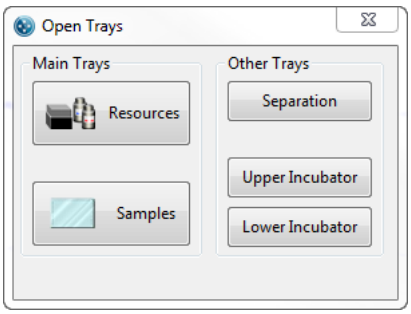

Cleanup
Make sure the separation tray troughs are clean before clicking "OK".
To clean the troughs click "Cancel" and use the "Open Trays" menu to access the separation tray troughs. Cleaning the troughs may require soaking them with distilled water.
If the troughs have already been cleaned, click "OK" to continue.
OK
Cancel

If the troughs in the separation tray are empty, click on **OK** and proceed with “Cleanup” on page 121.

If Running Buffer is present in the separation tray, click on **Cancel** and manually remove the buffer. Evaporation of the Running Buffer will result in a highly viscous residue which the automatic cleaning feature cannot remove.

To remove the Running Buffer:

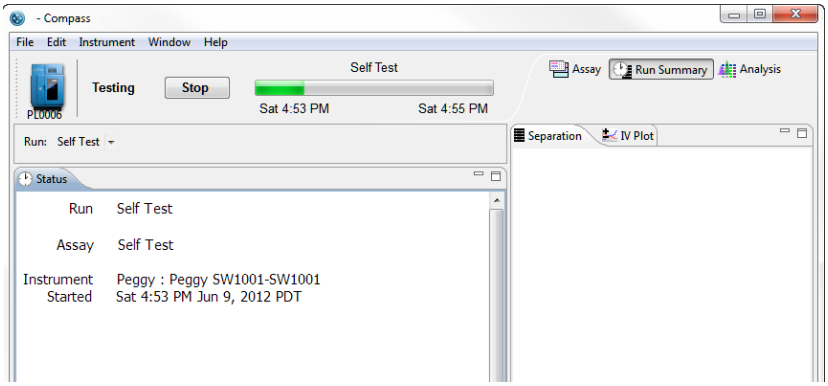
1. Select **Instrument** and click **Open Trays**.



2. Click **Separation** to open the separation tray.
3. Add 800 µL of deionized water to the troughs in the separation tray and soak for 20 minutes.
4. Remove the water by either aspirating with a pipette or with the vacuum wand located on the inside of Peggy's left door.
5. Repeat the steps above until the Running Buffer or residues are completely removed.
6. To complete the cleaning process, select **Instrument** and click **Cleanup**.

Self Test

Peggy can perform a series of self tests to check for proper instrument performance. To start the test, select **Instrument** and click **Self Test**. The test takes approximately two minutes.



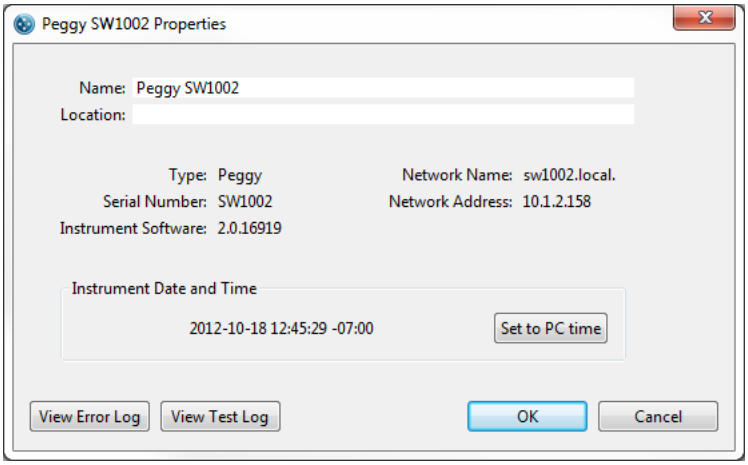
NOTE: ProteinSimple recommends performing the self test prior to starting a run.

To view the test log at completion of the test, select **Instrument**, click **Properties** and click **View Test Log**. See "Self Test Logs" on page 128 for more information.

Viewing and Changing System Properties

Select **Instrument** and click **Properties** to display system properties which include:

- Name
- Location
- Type
- Serial number
- Instrument software version (firmware)
- Network name and address
- Date and time of the instrument clock

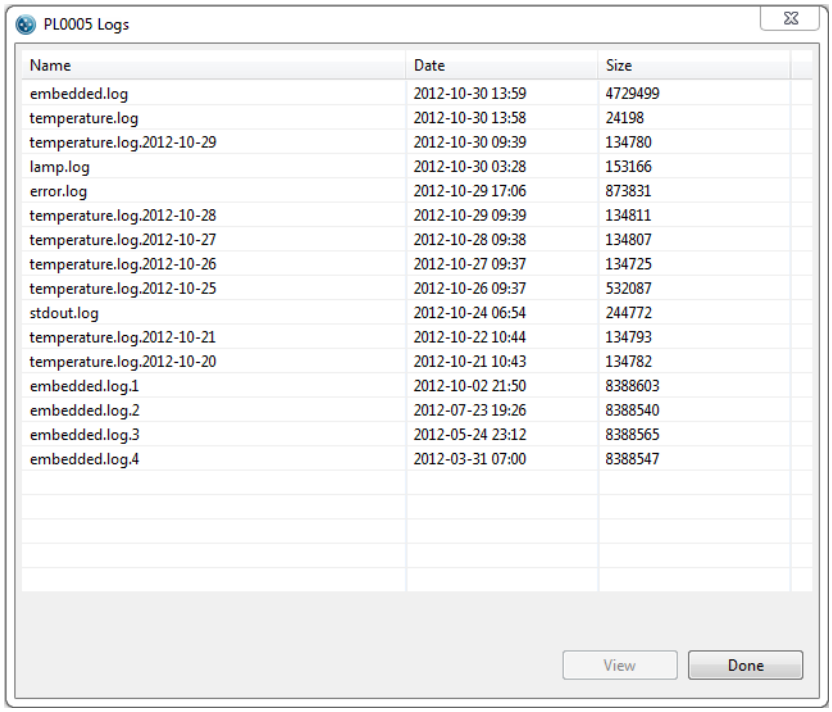


- **To change system name or location** - click in the name or location boxes and enter the new information.
- **To sync the instrument clock with the computer** - click **Set to PC time**.

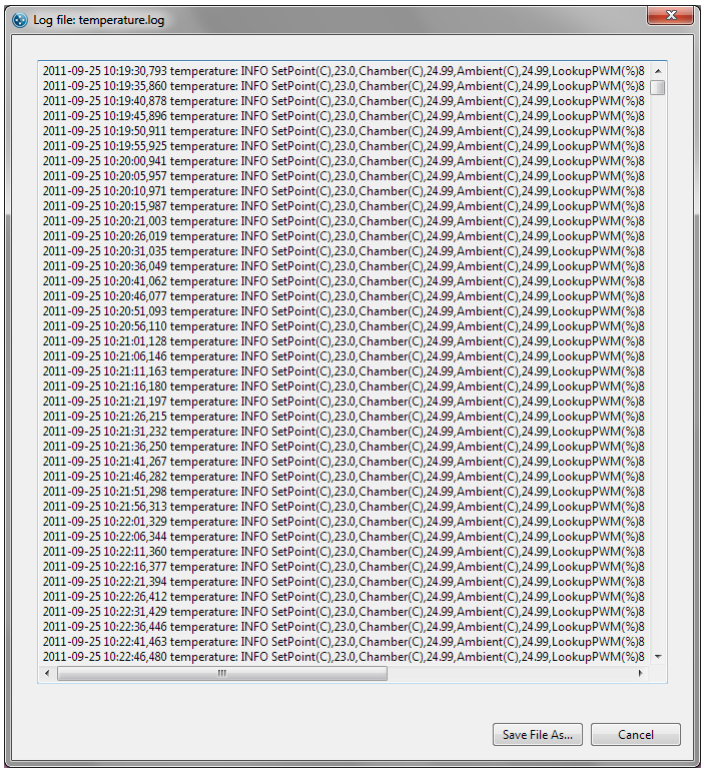
Viewing Log Files

Error Logs

1. Select **Instrument** in the main menu and click **Properties** to display system properties.
2. Click **View Error Log**. A list of system logs will display:



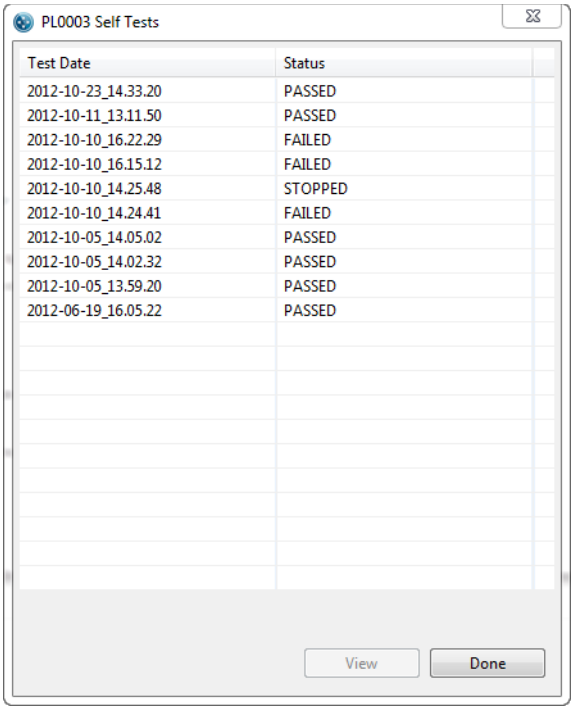
3. Select a log file and click **View**. The log details will display:



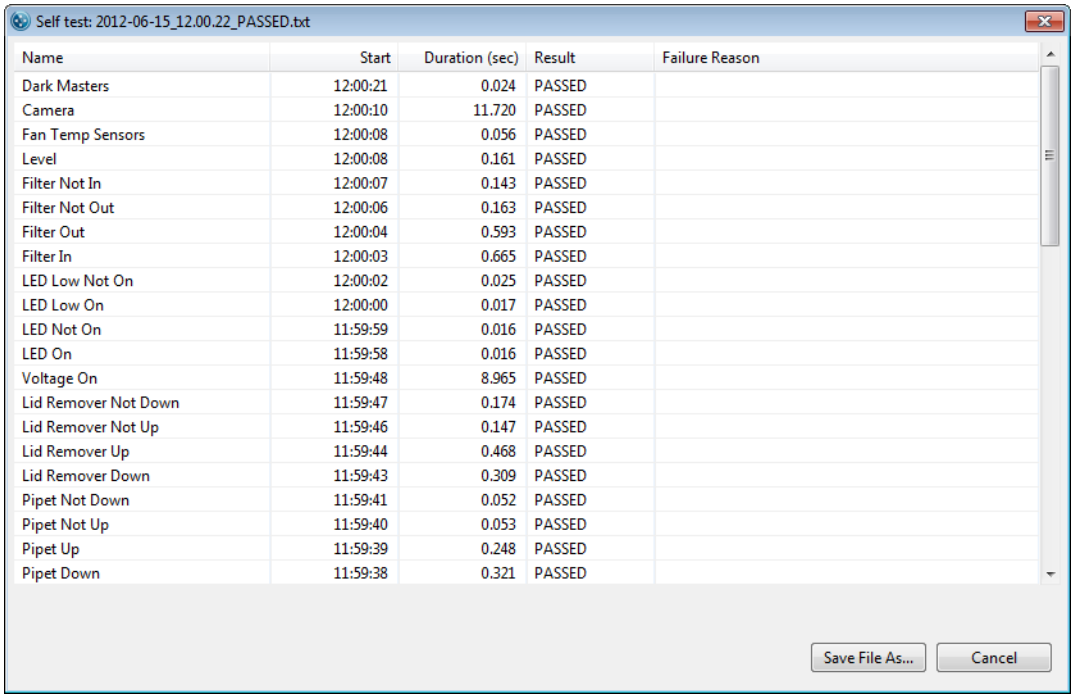
4. Click **Save File As** to save a copy of the log file.

Self Test Logs

1. Select **Instrument** in the main menu and click **Properties** to display system properties.
2. Click **View Test Log**. A list of self test logs will display:



3. Select a log file and click **View**. The individual test details will display:


Self test: 2012-06-15_12.00.22_PASSED.txt
Name Start Duration (sec) Result Failure Reason
Dark Masters 12:00:21 0.024 PASSED
Camera 12:00:10 11.720 PASSED
Fan Temp Sensors 12:00:08 0.056 PASSED
Level 12:00:08 0.161 PASSED
Filter Not In 12:00:07 0.143 PASSED
Filter Not Out 12:00:06 0.163 PASSED
Filter Out 12:00:04 0.593 PASSED
Filter In 12:00:03 0.665 PASSED
LED Low Not On 12:00:02 0.025 PASSED
LED Low On 12:00:00 0.017 PASSED
LED Not On 11:59:59 0.016 PASSED
LED On 11:59:58 0.016 PASSED
Voltage On 11:59:48 8.965 PASSED
Lid Remover Not Down 11:59:47 0.174 PASSED
Lid Remover Not Up 11:59:46 0.147 PASSED
Lid Remover Up 11:59:44 0.468 PASSED
Lid Remover Down 11:59:43 0.309 PASSED
Pipet Not Down 11:59:41 0.052 PASSED
Pipet Not Up 11:59:40 0.053 PASSED
Pipet Up 11:59:39 0.248 PASSED
Pipet Down 11:59:38 0.321 PASSED
Save File As...
Cancel

4. Click **Save File As** to save a copy of the log file.

Peggy's Status Modes

The instrument status bar displays status, buttons and progress bars depending on what Peggy is doing.

- **Ready/New Run button** - Peggy is ready but an assay is not loaded. Click **New Run** to open an assay.
- **Ready/Start button** - Peggy is ready and an assay is loaded. Click **Start** to begin a run.
- **Not Ready/Clean button** - Peggy is not ready and must perform system cleaning. Click **Clean** to start the cleaning protocol.
- **Not Ready/Reset button** - Peggy is not ready and must reinitialize. Click **Reset** to start the initialization protocol.
- **Running/Stop button** - Peggy is running an assay. The run name, time the run started and when it will complete display in the run progress bar. Click **Stop** to stop the run.
- **Cleaning/button not active** - Peggy is running a cleaning protocol. The time the cleaning protocol started and when it will complete display in the run progress bar.
- **Error/Reset button** - An error has occurred. Go to the **Status** window in the **Run Summary** screen to view details. When the source of the error is corrected, click **Reset**.

Chapter 8:

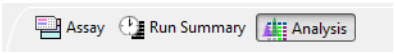
Size Assay Data Analysis

Chapter Overview

- Analysis Screen Overview
- Opening Run Files
- How Run Data is Displayed in the Analysis Screen
- Viewing Run Data
- Compass Run Data Notifications and Warnings
- Checking Your Results
- Group Statistics
- Copying Data Views and Results Tables
- Exporting Run Files
- Changing Sample Protein Identification
- Changing the Virtual Blot View
- Changing the Electropherogram View
- Closing Run Files
- Compass Analysis Settings Overview
- Advanced Analysis Settings
- Images Analysis Settings
- Peak Fit Analysis Settings
- Peak Names Settings
- Standards Settings
- Importing and Exporting Analysis Settings

Analysis Screen Overview

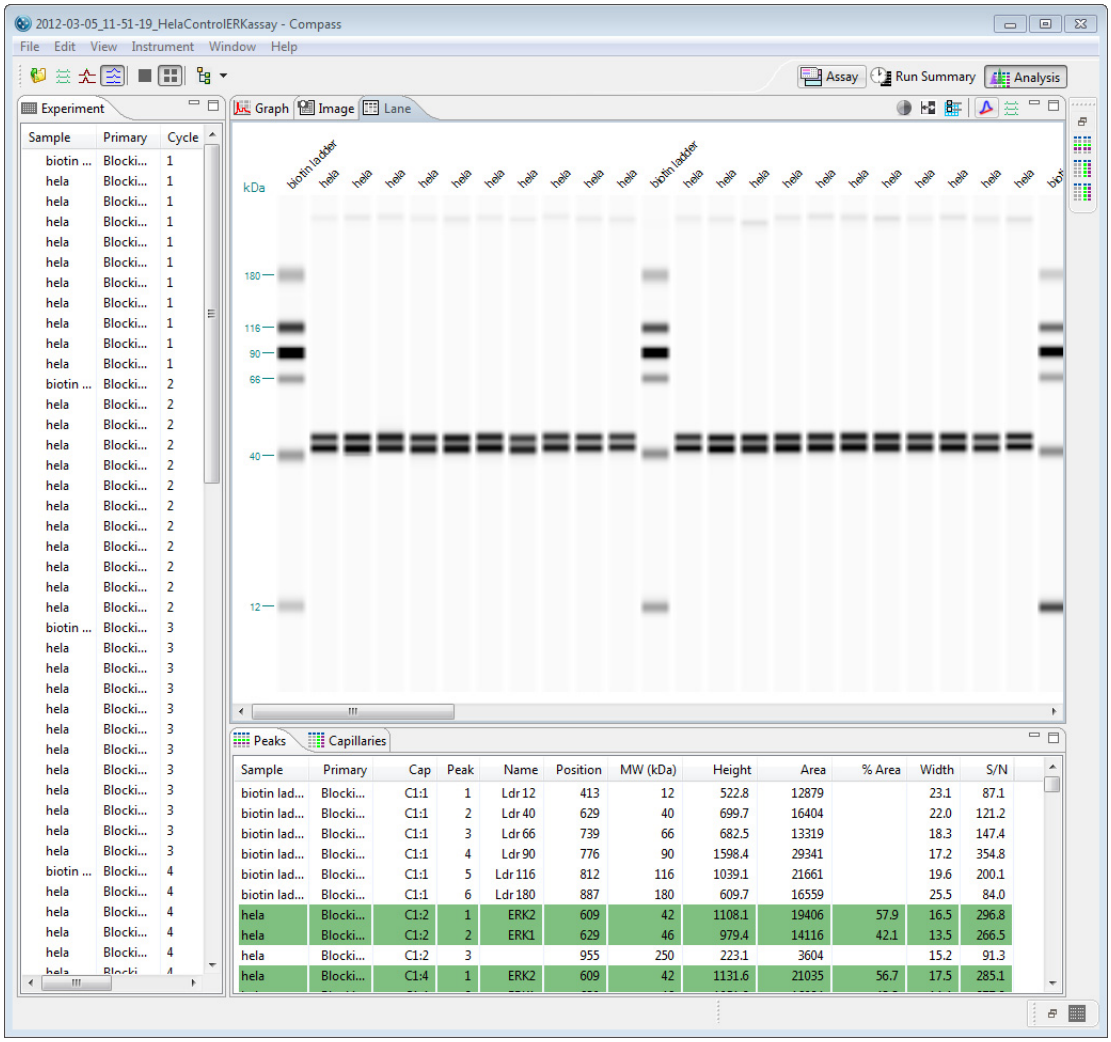
The Analysis screen is used to view run data including electropherograms, capillary images, lane view data and tabulated results. Any post-run analysis is also done in this screen. To access this screen, click **Analysis** in the screen tab:



Analysis Screen Panes

The Analysis screen has six panes, each displays the following data for up to 96 capillaries per experimental run:

- **Experiment** - Lists the assay protocol steps and assay template information.
- **Graph** - Displays electropherogram data for immunodetected sample proteins, fluorescent standards or capillary registrations.
- **Image** - Displays a 12-capillary image of the separated immunodetected sample proteins, fluorescent standards or capillary registrations.
- **Lane** - Displays data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results.
- **Peaks** - Lists the tabulated results for immunodetected sample proteins and information for identified fluorescent standards and capillary registrations.
- **Capillaries** - Displays a list of the immunodetected sample proteins Compass named automatically using the user-defined peak name analysis parameters.


2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit View Instrument Window Help
Assay
Run Summary
Analysis
Experiment
Graph
Image
Lane
Sample Primary Cycle
kDa
biotin ladder
hela
180
116
90
66
40
12
Peaks
Capillaries
Sample Primary Cap Peak Name Position MW (kDa) Height Area % Area Width S/N
biotin lad... Blocki... C1:1 1 Ldr 12 413 12 522.8 12879 23.1 87.1
biotin lad... Blocki... C1:1 2 Ldr 40 629 40 699.7 16404 22.0 121.2
biotin lad... Blocki... C1:1 3 Ldr 66 739 66 682.5 13319 18.3 147.4
biotin lad... Blocki... C1:1 4 Ldr 90 776 90 1598.4 29341 17.2 354.8
biotin lad... Blocki... C1:1 5 Ldr 116 812 116 1039.1 21661 19.6 200.1
biotin lad... Blocki... C1:1 6 Ldr 180 887 180 609.7 16559 25.5 84.0
hela Blocki... C1:2 1 ERK2 609 42 1108.1 19406 57.9 16.5 296.8
hela Blocki... C1:2 2 ERK1 629 46 979.4 14116 42.1 13.5 266.5
hela Blocki... C1:2 3 955 250 223.1 3604 15.2 91.3
hela Blocki... C1:4 1 ERK2 609 42 1131.6 21035 56.7 17.5 285.1

NOTE: The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

Software Menus Active in the Analysis Screen

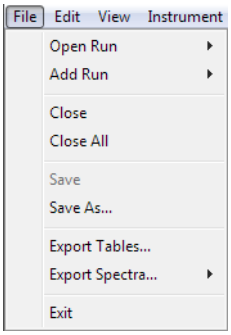
The following software menus are available:

- File
- Edit
- View
- Instrument (when Compass is connected to Peggy)
- Window
- Help

The File, Edit and View menu options specific to the Analysis screen are described next.

File Menu

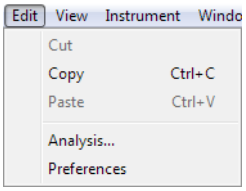
The following File menu options are active:



- **Open Run** - Opens a run file.
- **Add Run** - Opens and views other run files in addition to those that are already open.
- **Close** - Closes the run file currently being viewed.
- **Close All** - Closes all open run files.
- **Save** - Saves changes to the open run file.
- **Save As** - Saves changes to the open run file under a different file name.
- **Export Tables** - Exports the results for all capillaries in the run in .txt format.
- **Export Spectra** - Exports the raw data traces for each capillary in the run in .txt or .cdf format.
- **Exit** - Closes Compass.

Edit Menu

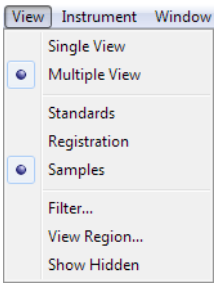
The following Edit menu options are active:



- **Copy** - Lets you copy data shown in the graph, lane, peaks or capillaries panes. See "Copying Data Views and Results Tables" on page 181 for more information.
- **Analysis** - Displays the analysis settings used to analyze the run data and lets you change them as needed. See "Compass Analysis Settings Overview" on page 219 for more information.
- **Preferences** - Lets you set and save custom preferences for data export, plot colors in the graph and Peggy's Twitter settings. See Chapter 10, *"Setting Custom User Preferences"* for more information.

View Menu

The following View menu options are active:



- **Single View** - Displays data in a per capillary (single) view format.
- **Multiple View** - Displays data in a per 12-capillary (multiple) view format.
- **Standards** - Lets you change the data view to show only the fluorescent standards.
- **Registration** - Lets you change the data view to show only the capillary registrations.
- **Samples** - Lets you change the data view to show only immunodetected sample proteins.
- **Filter** - Lets you display data only for specific capillaries or named proteins.
- **View Region** - Lets you change the molecular weight (x-axis) range of the data displayed.
- **Show Hidden**- Shows capillaries that are hidden from the data view.

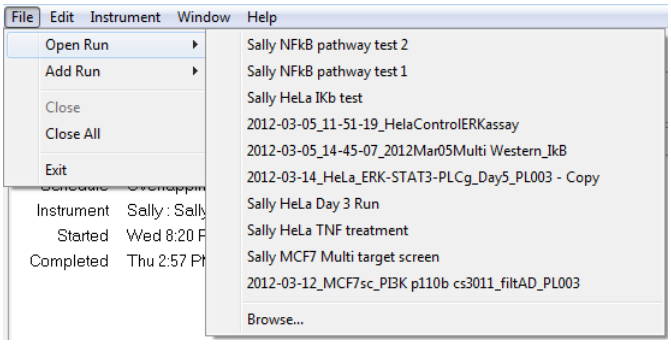
Opening Run Files

You can open one or multiple run files at a time to compare data between runs.

Opening One Run File

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

1. Select **File** in the main menu and click **Open Run**.

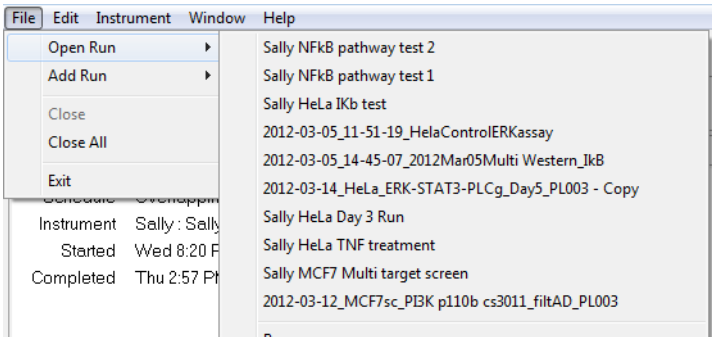


2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

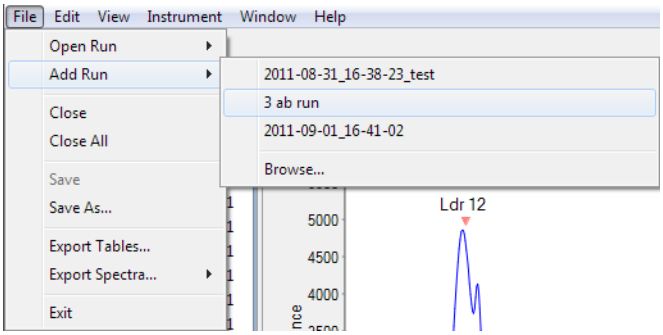
Opening Multiple Run Files

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

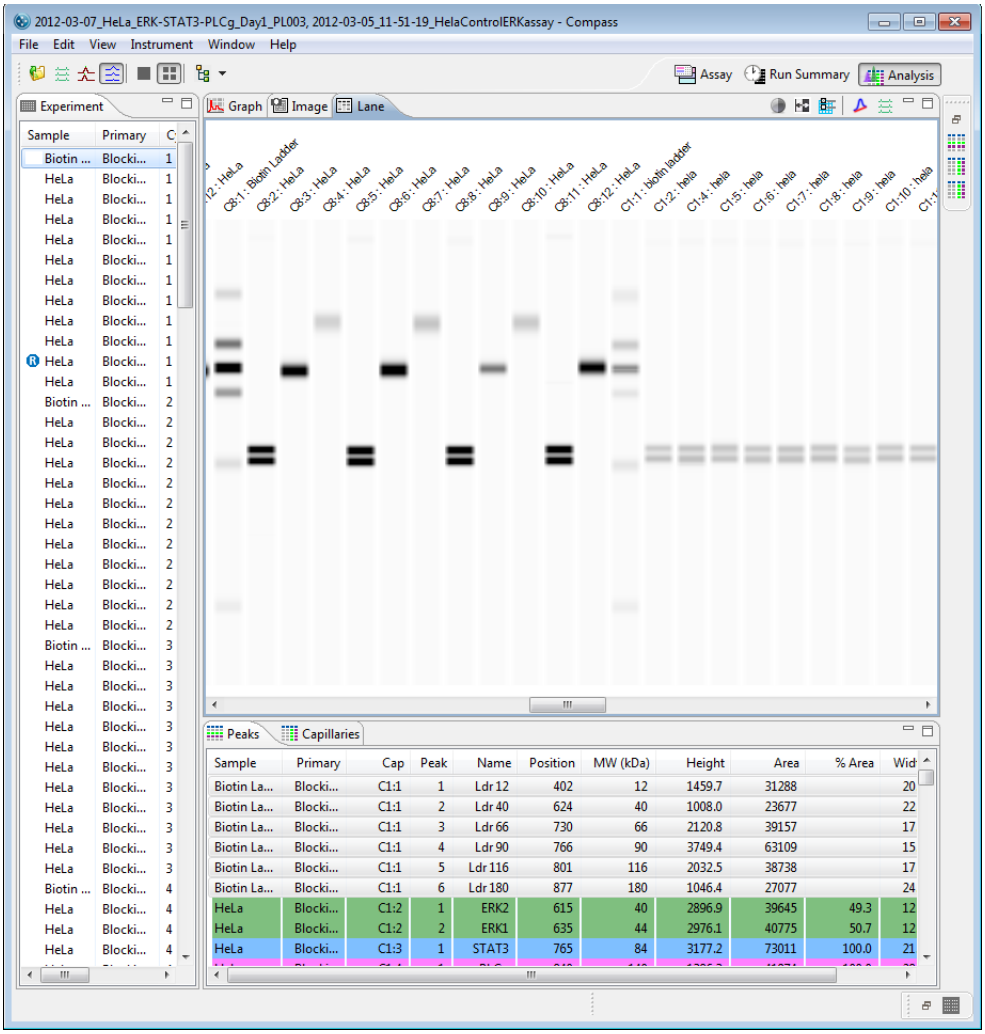
1. To open the first run file, select **File** in the main menu and click **Open Run**.



2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
3. To open another run file, select **File** in the main menu and click **Add Run**.



4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file. When a run is added, its data will append to the open run file, and will display as a second set of up to 96 capillaries in all screen panes. The second run file name will also appear in the Compass title bar:



Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Wid
Biotin La...	Blocki...	C1:1	1	Ldr 12	402	12	1459.7	31288		20
Biotin La...	Blocki...	C1:1	2	Ldr 40	624	40	1008.0	23677		22
Biotin La...	Blocki...	C1:1	3	Ldr 66	730	66	2120.8	39157		17
Biotin La...	Blocki...	C1:1	4	Ldr 90	766	90	3749.4	63109		15
Biotin La...	Blocki...	C1:1	5	Ldr 116	801	116	2032.5	38738		17
Biotin La...	Blocki...	C1:1	6	Ldr 180	877	180	1046.4	27077		24
HeLa	Blocki...	C1:2	1	ERK2	615	40	2896.9	39645	49.3	12
HeLa	Blocki...	C1:2	2	ERK1	635	44	2976.1	40775	50.7	12
HeLa	Blocki...	C1:3	1	STAT3	765	84	3177.2	73011	100.0	21

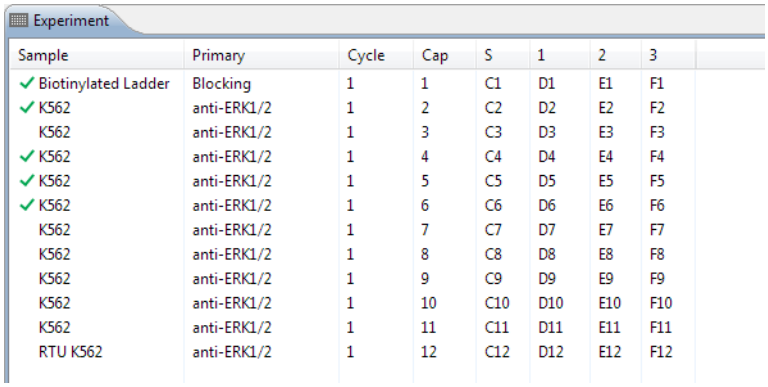
5. Repeat the last two steps to open additional runs.

How Run Data is Displayed in the Analysis Screen

Data in the run file is compartmentalized for easy review in one of six panes in the Analysis screen.

Experiment Pane: Assay and Capillary Information

The experiment pane displays assay and capillary information for each of the 96 capillaries in the run. A maximized view of the experiment pane is shown below.



Experiment

Sample	Primary	Cycle	Cap	S	1	2	3
✓ Biotinylated Ladder	Blocking	1	1	C1	D1	E1	F1
✓ K562	anti-ERK1/2	1	2	C2	D2	E2	F2
K562	anti-ERK1/2	1	3	C3	D3	E3	F3
✓ K562	anti-ERK1/2	1	4	C4	D4	E4	F4
✓ K562	anti-ERK1/2	1	5	C5	D5	E5	F5
✓ K562	anti-ERK1/2	1	6	C6	D6	E6	F6
K562	anti-ERK1/2	1	7	C7	D7	E7	F7
K562	anti-ERK1/2	1	8	C8	D8	E8	F8
K562	anti-ERK1/2	1	9	C9	D9	E9	F9
K562	anti-ERK1/2	1	10	C10	D10	E10	F10
K562	anti-ERK1/2	1	11	C11	D11	E11	F11
RTU K562	anti-ERK1/2	1	12	C12	D12	E12	F12

- **To view all columns** - Click the **Experiment** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Experiment** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Experiment pane column descriptions for the Peggy Size default assay are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.

NOTE: Data notification icons will display in the sample column if Compass detected a potential analysis issue or data was manually modified by the user. For more information see "Compass Run Data Notifications and Warnings" on page 162.

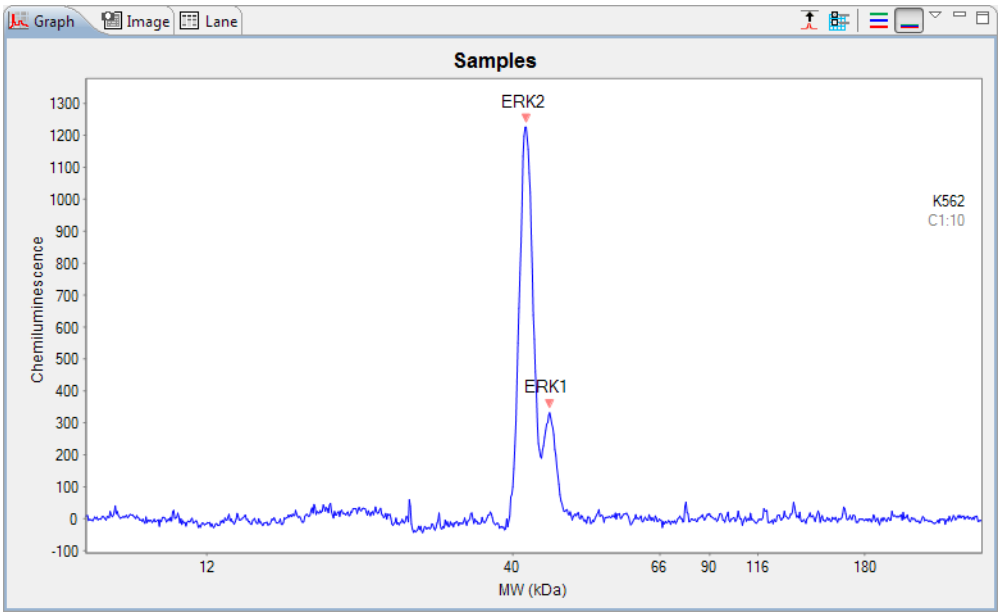
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Cycle** - Run cycle number. There are 12 capillaries in one cycle.

NOTE: Peggy can run up to eight cycles (12 capillaries per cycle) in an experiment. Each cycle is designated in the Experiment summary.

- **Cap** - Capillary number.
- **S** - Well on the assay plate used for sample.
- **1** - Well on the assay plate used for blocking buffer (Antibody Diluent).
- **2** - Well on the assay plate used for primary antibody.
- **3** - Well on the assay plate used for secondary HRP-conjugate.

Graph Pane: Electropherogram Data

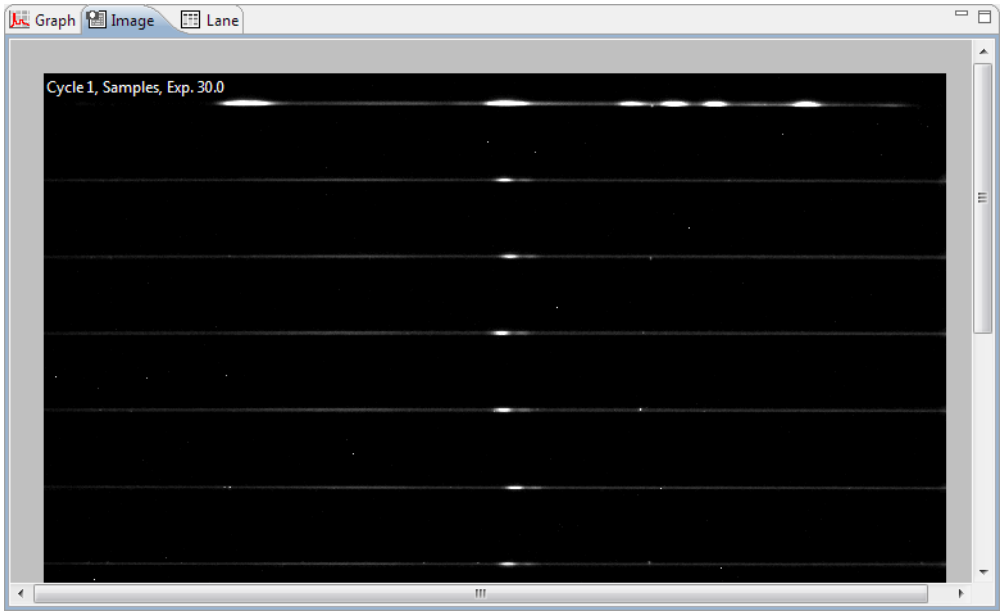
Click the **Graph** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations. Data for samples is shown in the following example, and immunodetected proteins are displayed as peaks:



More Graph view options will be described in more detail in "Changing the Electropherogram View" on page 196.

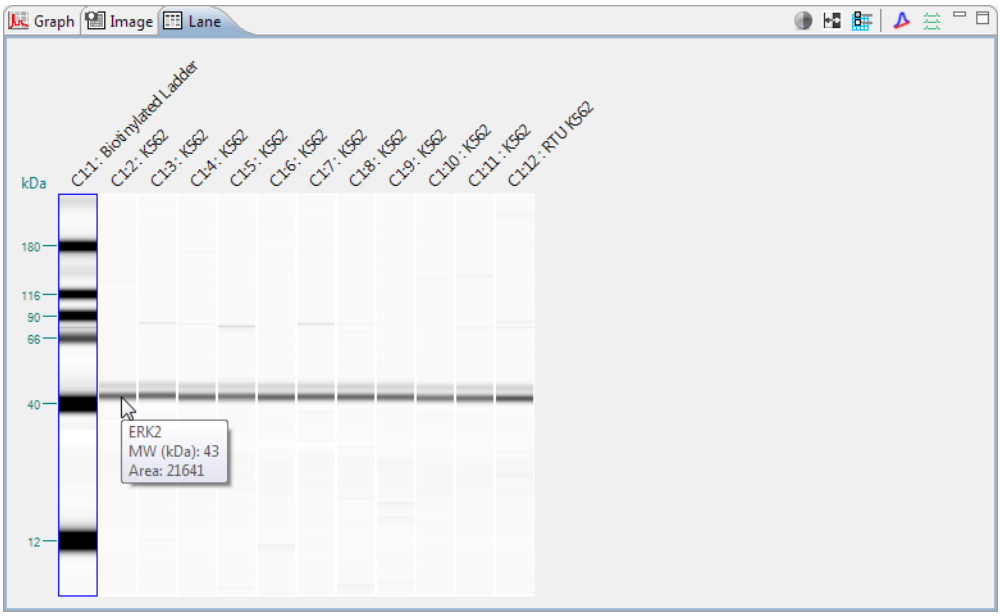
Image Pane: Capillary Separation Image Data

Click the **Image** tab to view final separation images of immunodetected sample proteins, fluorescent standards or capillary registrations for up to 96 capillaries per experimental run. Image data for samples is shown in the following example:



Lane Pane: Virtual Blot-Like Image Data

Click the **Lane** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results. Data for samples in the lane view is shown in the following example, and immunodetected proteins are displayed as bands.



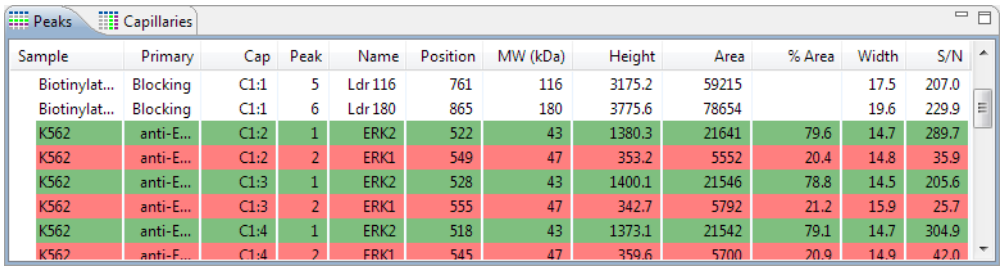
To view information for a band, roll the mouse over a band until the info box appears.

NOTE: The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

Lane data displayed in the virtual blot is automatically aligned by Compass. To view raw, unaligned lane data and learn more about virtual blot viewing options, see "Changing the Virtual Blot View" on page 190.

Peaks Pane: Calculated Results

Click the **Peaks** tab to view tabulated results for immunodetected sample proteins, fluorescent standards or capillary registrations. Each row in the table shows the individual results for each peak detected in each capillary. Data for samples in the peaks table is shown in the following example:



Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N
Biotinylat...	Blocking	C1:1	5	Ldr 116	761	116	3175.2	59215		17.5	207.0
Biotinylat...	Blocking	C1:1	6	Ldr 180	865	180	3775.6	78654		19.6	229.9
K562	anti-E...	C1:2	1	ERK2	522	43	1380.3	21641	79.6	14.7	289.7
K562	anti-E...	C1:2	2	ERK1	549	47	353.2	5552	20.4	14.8	35.9
K562	anti-E...	C1:3	1	ERK2	528	43	1400.1	21546	78.8	14.5	205.6
K562	anti-E...	C1:3	2	ERK1	555	47	342.7	5792	21.2	15.9	25.7
K562	anti-E...	C1:4	1	ERK2	518	43	1373.1	21542	79.1	14.7	304.9
K562	anti-E...	C1:4	2	ERK1	545	47	359.6	5700	20.9	14.9	42.0

NOTES:
Peaks that Compass names automatically using the user-defined peak name analysis parameters are color-coded.

The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

- **To view all rows** - Click the **Peaks** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Peaks** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Peak table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Cap** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

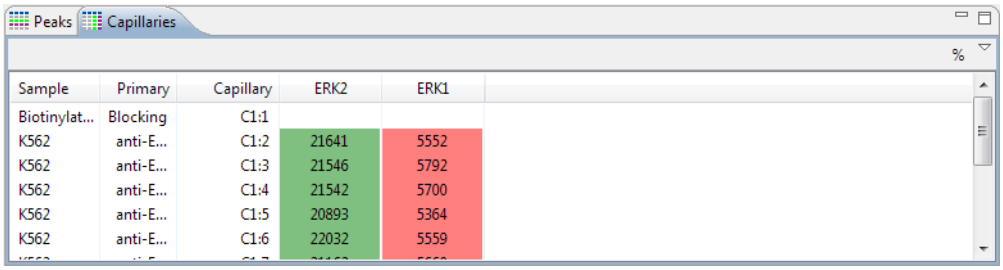
NOTE: Peggy runs up to eight cycles in an experiment, 12 capillaries at a time. Capillary and cycle numbers are displayed in the Experiment tab.

- **Peak** - Peak number. Peaks are numbered in order of detection.

- **Name** - Peak name. Displays peaks that Compass named automatically using the user-defined peak name analysis parameters. Cells will be blank if Compass was not able to name the peak or if naming parameters were not entered.
- **Position** - Displays the pixel position of a peak in the image.
- **MW (kDa)** - Displays the calculated molecular weight in kDa for the peak (shown for sample data only).
- **Height** - Displays the calculated peak height.
- **Area** - Displays the calculated peak area (shown for sample data only).
- **% Area** - Displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100 (shown for named peak sample data only).
- **Width** - Displays the calculated peak width (shown for sample data only).
- **S/N** - Displays the calculated signal to noise ratio for the peak (shown for sample data only).

Capillaries Pane: User-Specified Peak Names

Click the **Capillaries** tab to view tabulated results for sample proteins associated with specific primary antibodies in the run data. Compass labels these sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary. Data for samples in the capillaries table is shown in the following example.



Sample	Primary	Capillary	ERK2	ERK1
Biotinylat...	Blocking	C1:1		
K562	anti-E...	C1:2	21641	5552
K562	anti-E...	C1:3	21546	5792
K562	anti-E...	C1:4	21542	5700
K562	anti-E...	C1:5	20893	5364
K562	anti-E...	C1:6	22032	5559

NOTES:
Peaks that Compass names automatically with user-defined peak name settings are color-coded.

Information displayed for fluorescent standards and capillary registration data will be for identified standards or registration peaks.

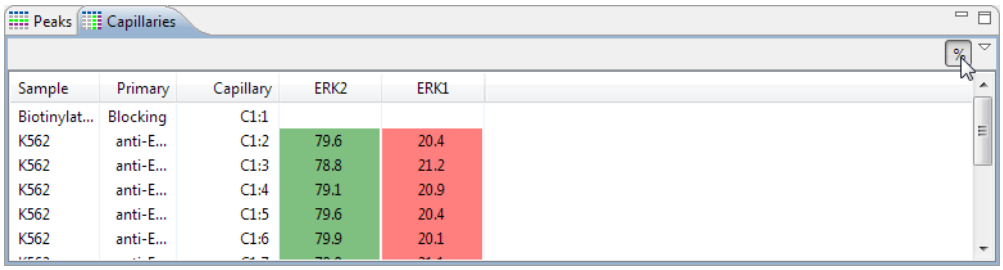
- **To view all rows** - Click the **Capillaries** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Capillaries** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Capillaries table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

NOTE: Peggy runs12 capillaries at a time in a cycle and is able to run up to eight cycles in an experiment. The Information on cycle and capillary number is displayed in the Experiment tab.

- **Peak Name Columns** - An individual column per peak name will display for every peak identified by name in the run data. Cells for capillaries in these columns will be blank if Compass did not find peaks automatically using the user-defined peak name analysis parameters (or none were entered).
  - **To view % area in the peak name columns** - Select **%** in the upper right corner of the pane. This displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100.



Sample	Primary	Capillary	ERK2	ERK1
Biotinylat...	Blocking	C1:1		
K562	anti-E...	C1:2	79.6	20.4
K562	anti-E...	C1:3	78.8	21.2
K562	anti-E...	C1:4	79.1	20.9
K562	anti-E...	C1:5	79.6	20.4
K562	anti-E...	C1:6	79.9	20.1

  - **To view peak area in the peak name columns (default)** - Deselect **%** in the upper right corner of the pane. This displays calculated peak area for the individual peak only.

Viewing Run Data

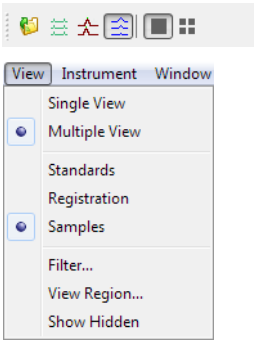
Each run file contains the following data for up to 96 capillaries:

- **Sample data** - For the immunodetected proteins in the sample.
- **Standards data** - For the fluorescent standards run with each sample.
- **Registration data** - For tracking capillaries as they are moved for various assay steps.

Data can be viewed for one, all, or individually selected capillaries.

Switching Between Sample, Standards and Registration Data Views

You can switch between viewing sample, standards and registration data in a run file using the View bar or View menu:



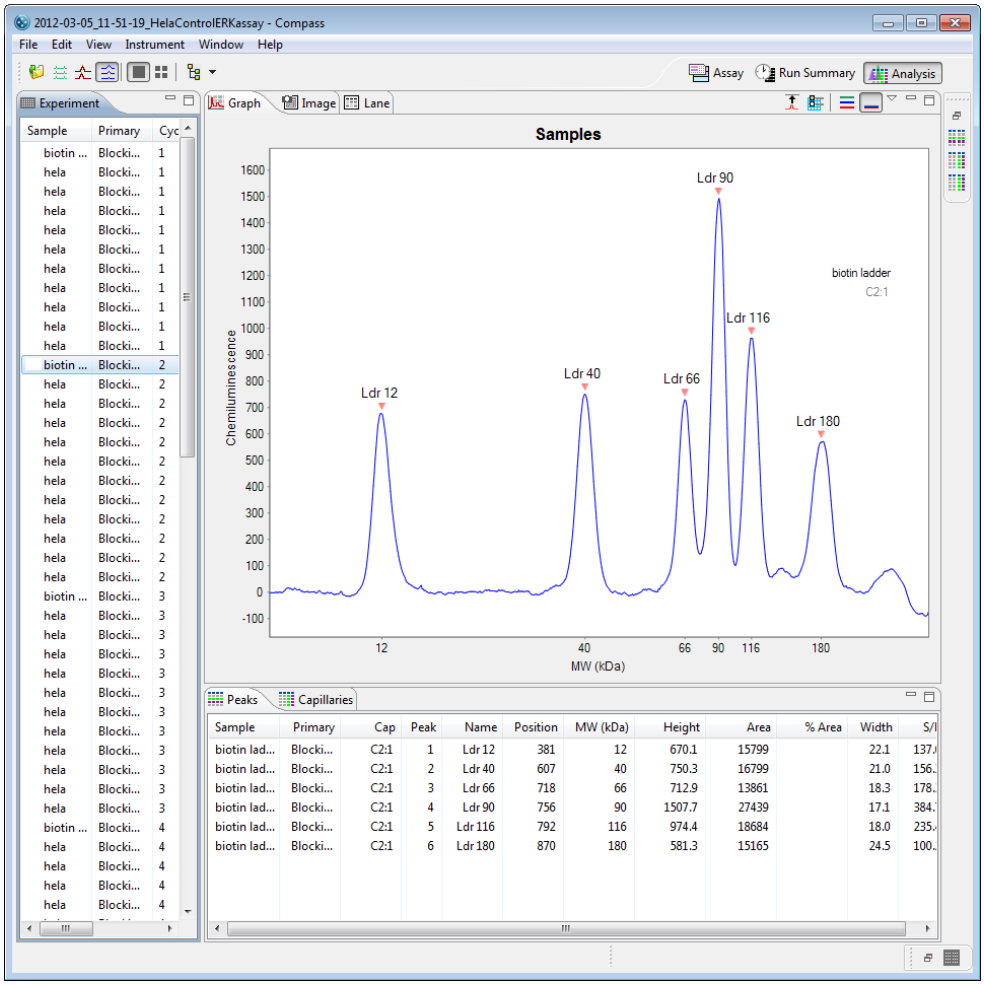
Data buttons in the View bar:

Show Standards

Show Registrations

Show Samples

- **To view sample data** - Click **Show Samples** in the View bar or select **View** in the main menu and click **Samples**:

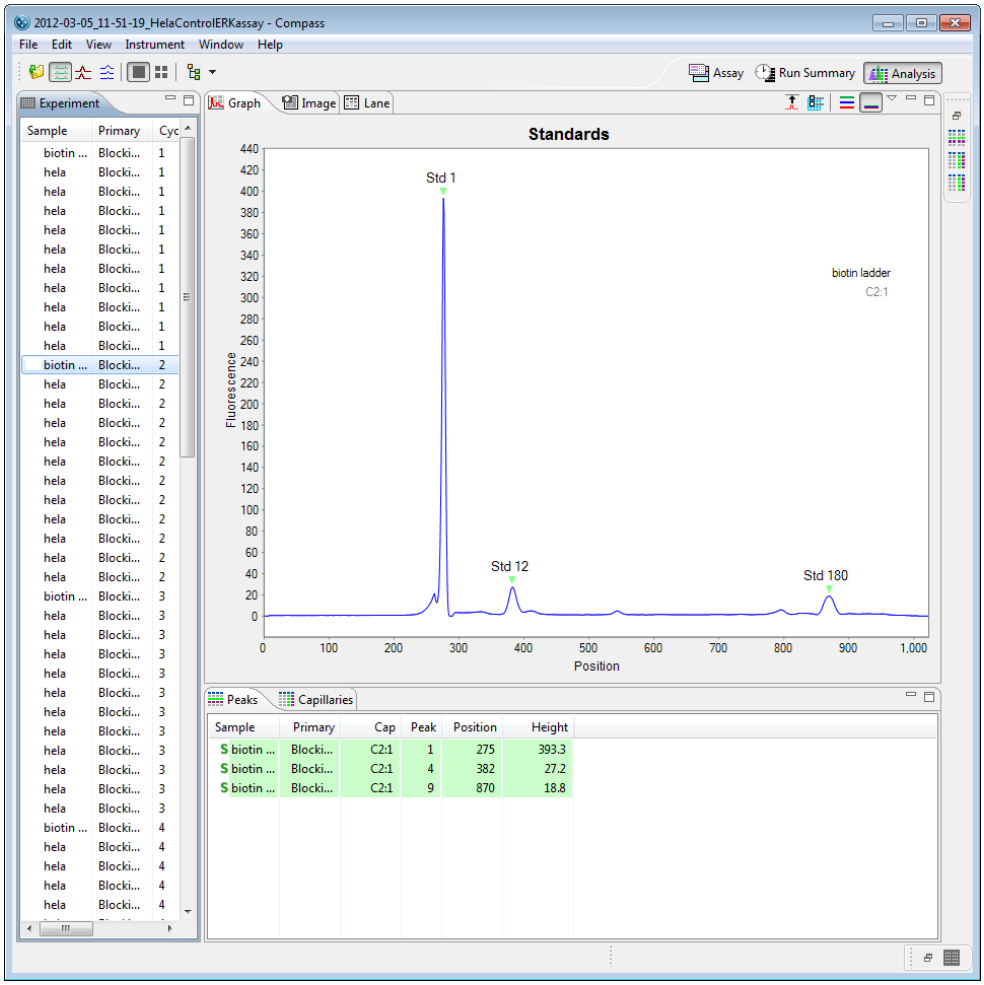


- Data in this view is for immunodetected sample proteins only.
- Graph view data displays electropherograms in chemiluminescence units (y-axis) and molecular weight in kDa (x-axis).
- Lane view data displays immunodetected sample proteins only.
- Image view data displays immunodetected sample proteins only.
- Results for each immunodetected protein are shown in the peaks and capillaries tables.

NOTE: The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

For information on checking and identifying sample peaks, see "Step 4 – Checking the Ladder" on page 169 or "Step 5 – Checking Samples" on page 171.

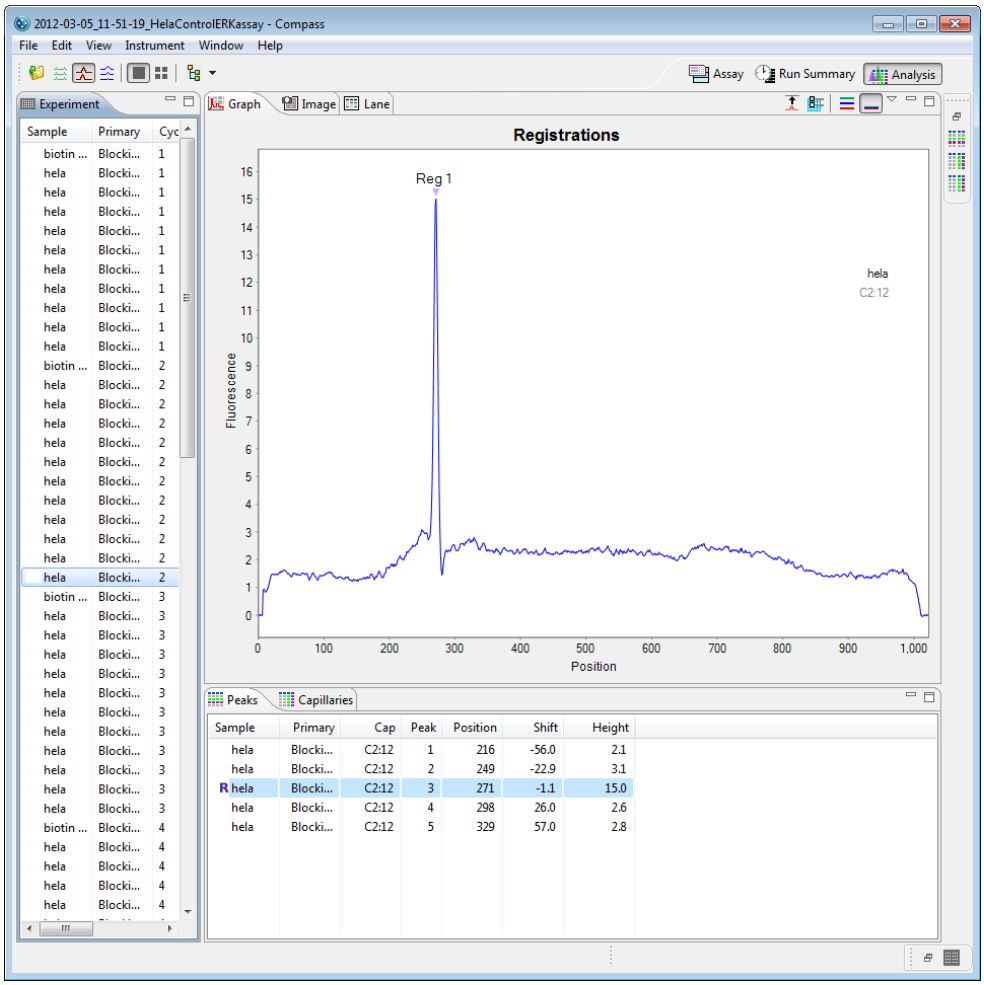
- **To view standards data** - Click **Show Standards** in the View bar or select **View** in the main menu and click **Standards**:



- Data in this view is for the fluorescent standards only. These standards are premixed with each sample prior to analysis.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays fluorescent standards only.
- Image view data displays fluorescent standards only.
- Standards are highlighted in both the peaks and capillaries tables and marked with an **S**.

For information on checking and identifying standards peaks, see "Step 2 – Checking Fluorescent Sizing Standards" on page 164.

- **To view registration data** - Click **Show Registrations** in the View bar or select **View** in the main menu and click **Registration**:



- Data in this view shows capillary registration only.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays capillary registration only.
- Image view data displays capillary registration only.
- Registrations are highlighted in both the peaks and capillaries tables and marked with an R.

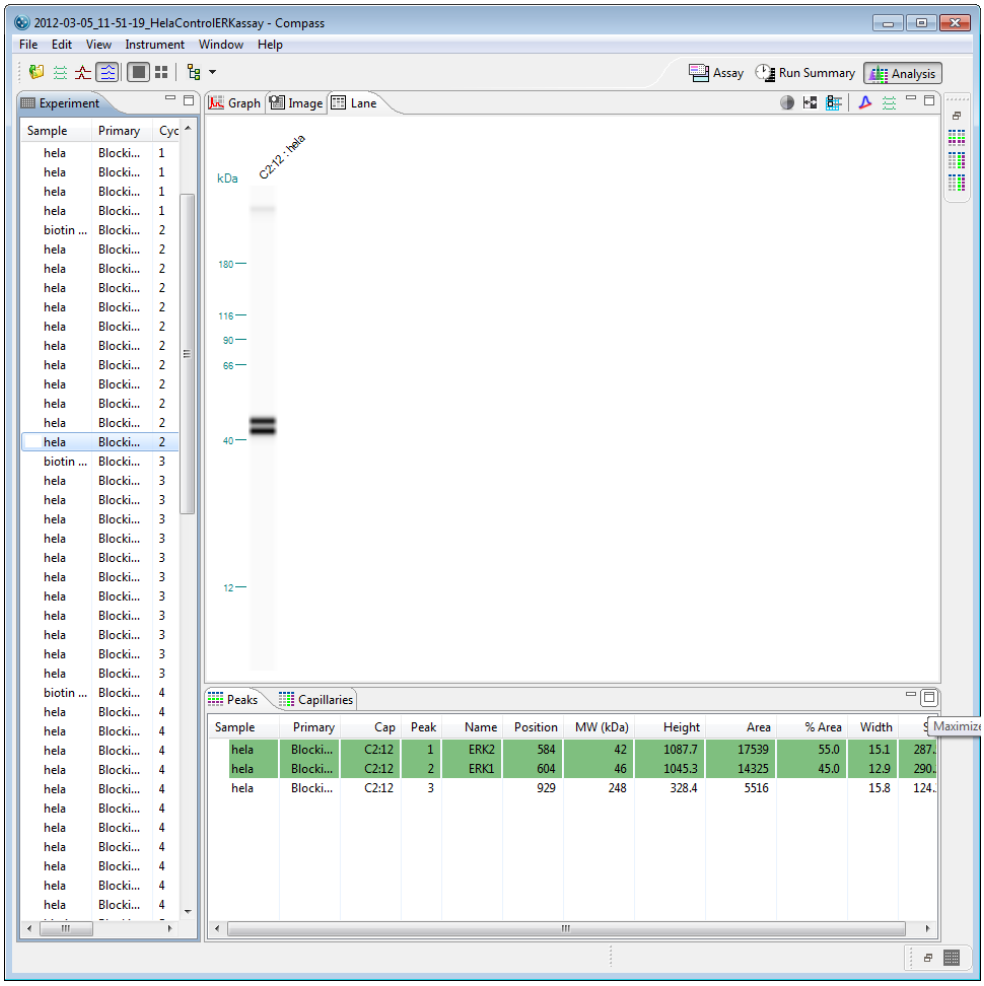
Because capillaries must be moved multiple times during the run, their positions are tracked to account for potential changes in imaging position as they are moved to and from the separation block. To do this, one of the standards is used as a capillary registration. An image of the standard used for registration is taken at the end of the separation step prior to chemiluminescent imaging. The position of this peak in the registration image is then compared to the position of the same standard peak in the standards image. The shift value listed in the peaks table is the change in pixel position between the two images. Compass data is then aligned to account for any changes in capillary position.

For information on checking and identifying registration peaks, see "Step 3 – Checking Capillary Registrations" on page 168.

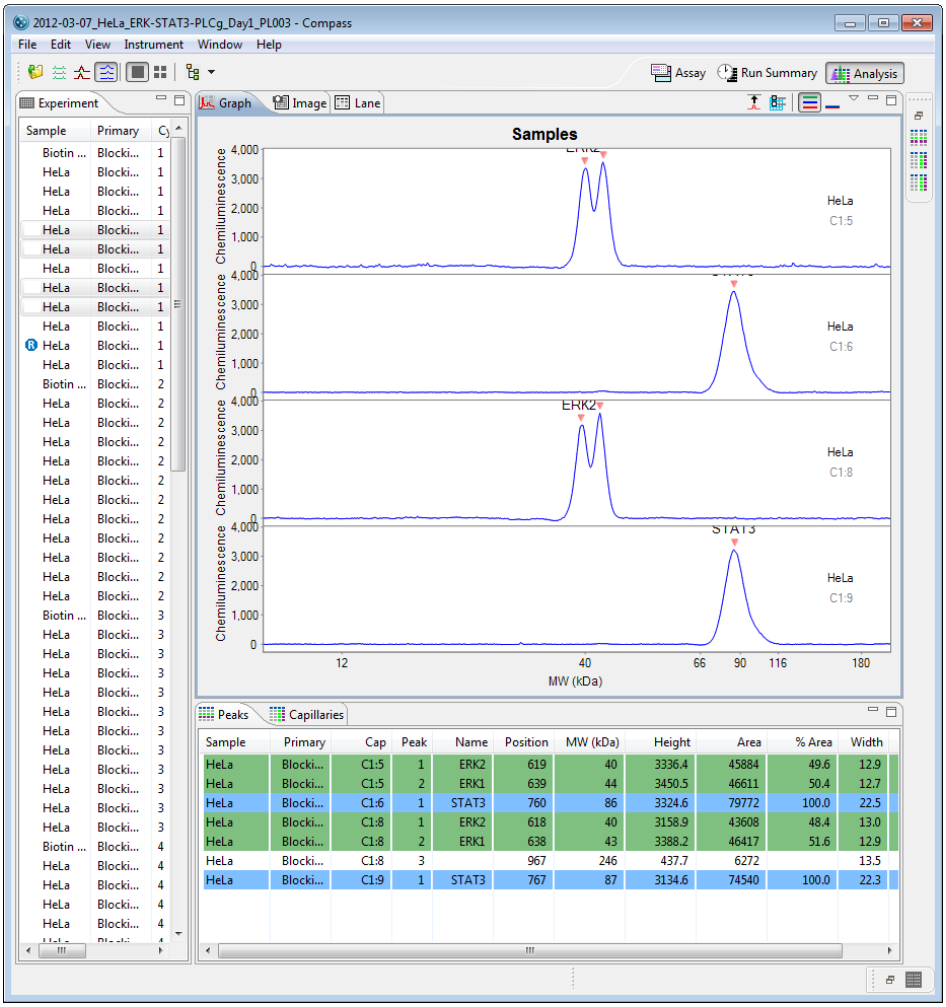
Selecting and Displaying Capillary Data

You can choose to view data from one, multiple, or all capillaries at once.

- **To look at data for one capillary** - Click a row in the experiment pane. Data for just the row selected will display in all data views and tables. The following example shows sample data displayed in the lane and peaks panes when one sample is selected:

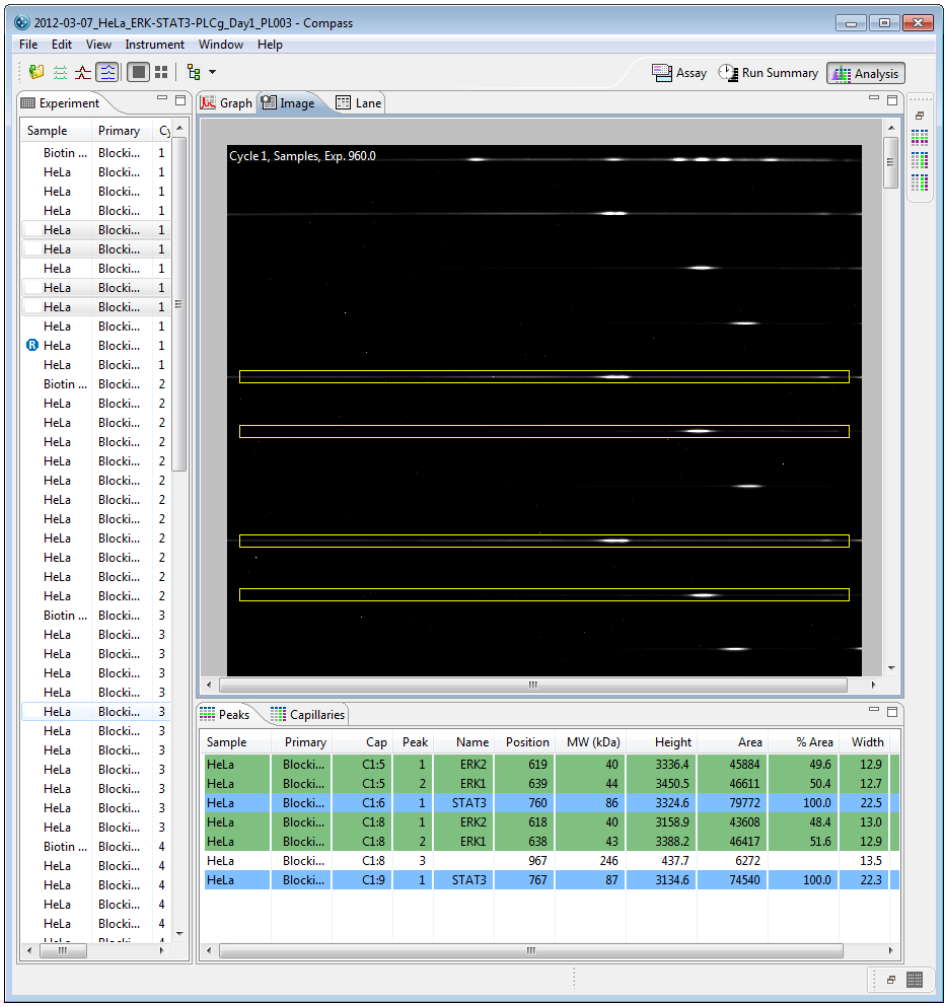

2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Experiment
Graph Image Lane
C2:12 : hela
kDa
180
116
90
66
40
12
Peaks Capillaries
Sample Primary Cap Peak Name Position MW (kDa) Height Area % Area Width
hela Blocki... C2:12 1 ERK2 584 42 1087.7 17539 55.0 15.1
hela Blocki... C2:12 2 ERK1 604 46 1045.3 14325 45.0 12.9
hela Blocki... C2:12 3 929 248 328.4 5516 15.8

- **To look at data for multiple non-sequential capillaries** - Hold the **Ctrl** key and select the rows you want to view in the experiment pane. Data for only the rows selected will display in all data views and results tables. The following example shows sample data displayed in the graph (in multiple view) and capillaries panes when multiple rows are selected:

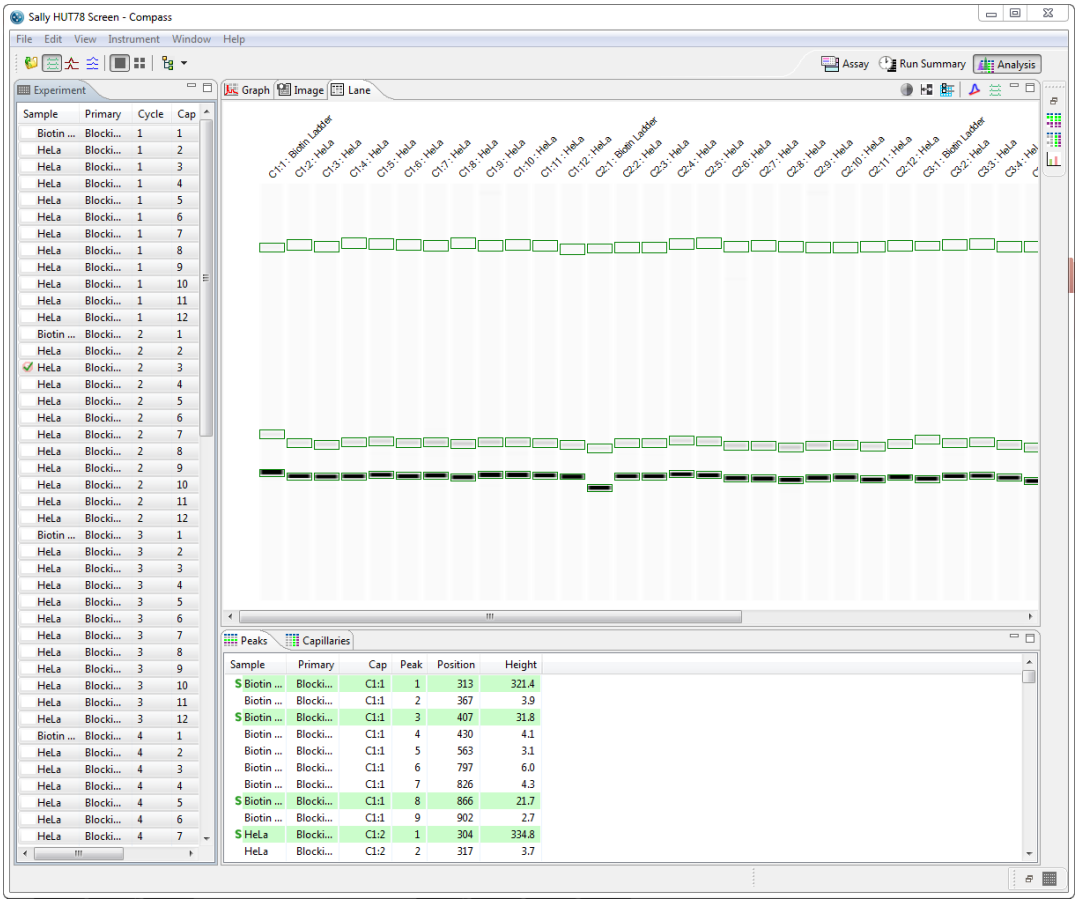


Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width
HeLa	Blocki...	C1:5	1	ERK2	619	40	3336.4	45884	49.6	12.9
HeLa	Blocki...	C1:5	2	ERK1	639	44	3450.5	46611	50.4	12.7
HeLa	Blocki...	C1:6	1	STAT3	760	86	3324.6	79772	100.0	22.5
HeLa	Blocki...	C1:8	1	ERK2	618	40	3158.9	43608	48.4	13.0
HeLa	Blocki...	C1:8	2	ERK1	638	43	3388.2	46417	51.6	12.9
HeLa	Blocki...	C1:8	3		967	246	437.7	6272		13.5
HeLa	Blocki...	C1:9	1	STAT3	767	87	3134.6	74540	100.0	22.3

- **To look at data for multiple sequential capillaries** - Hold the **Shift** key and select the first and last rows you want to view in the experiment pane. Data for only the selected rows will display in all data views and results tables. The following example shows standards data displayed in the image and peaks panes when multiple sequential rows are selected:

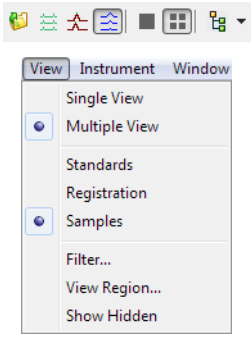


- **To look at data for all capillaries** - Hold the **Shift** key and select the first and last rows in the experiment pane. Data for all rows will display in all data views and results tables. The following example shows standards data displayed in the lane and peaks panes when all rows are selected. The pane scroll bars can be used to view all 8 cycles:



Switching Between Single and Multiple Views of the Capillaries

You can switch between displaying run data in the graph, image and lane panes in a per capillary (single) format or up to 96 capillary format. This view is selected in the View bar or the View menu.

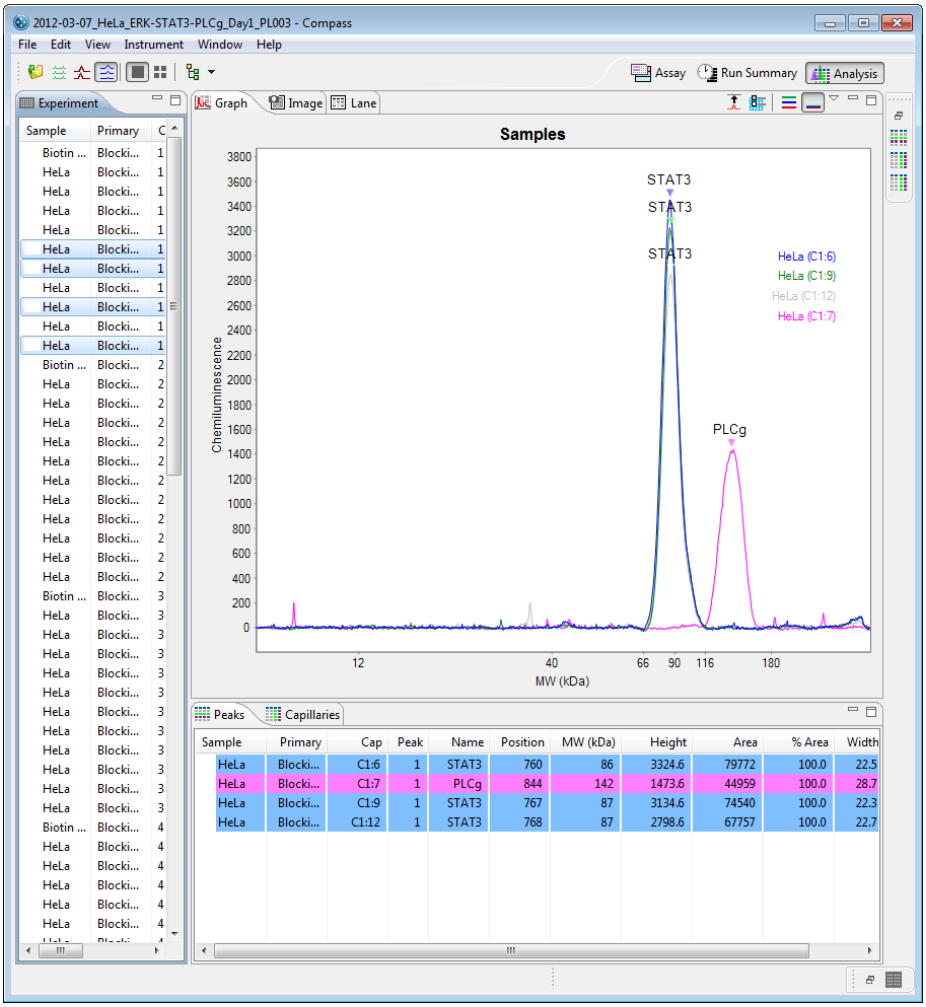


Capillary view buttons in the View bar:

Single View

Multiple View

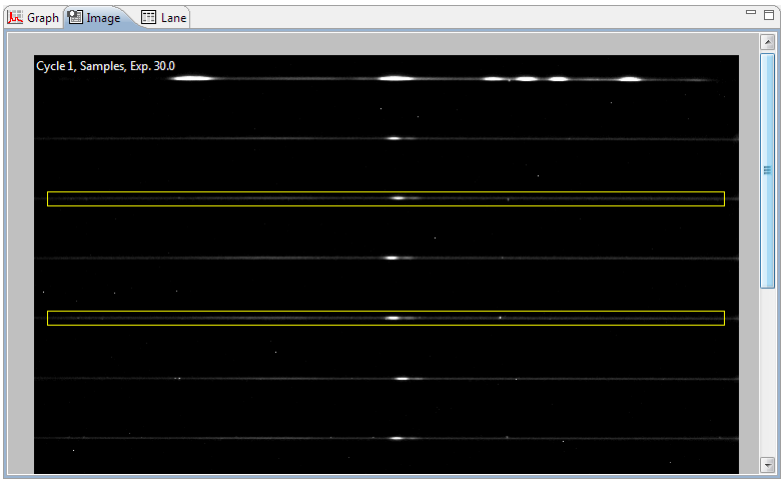
- **To view data in a per capillary format** - Click **Single View** in the View bar or select **View** in the main menu and click **Single View**:



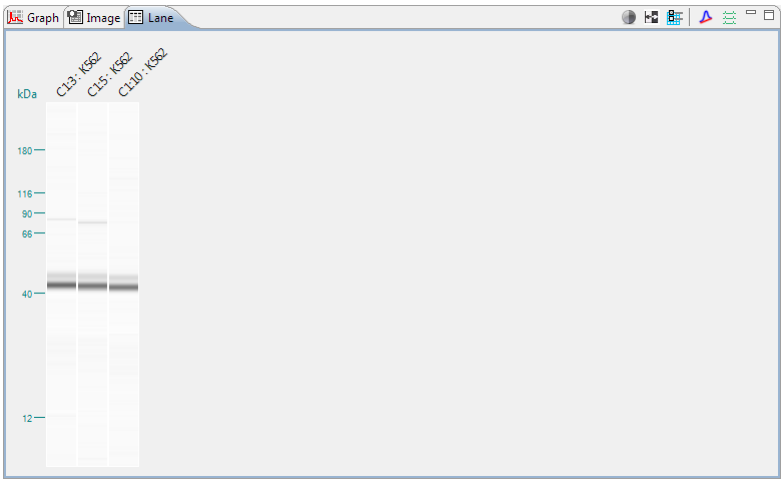
Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width
HeLa	Blocki...	C1:6	1	STAT3	760	86	3324.6	79772	100.0	22.5
HeLa	Blocki...	C1:7	1	PLCg	844	142	1473.6	44959	100.0	28.7
HeLa	Blocki...	C1:9	1	STAT3	767	87	3134.6	74540	100.0	22.3
HeLa	Blocki...	C1:12	1	STAT3	768	87	2798.6	67757	100.0	22.7

Data for the row(s) selected in the experiment pane will display as follows:

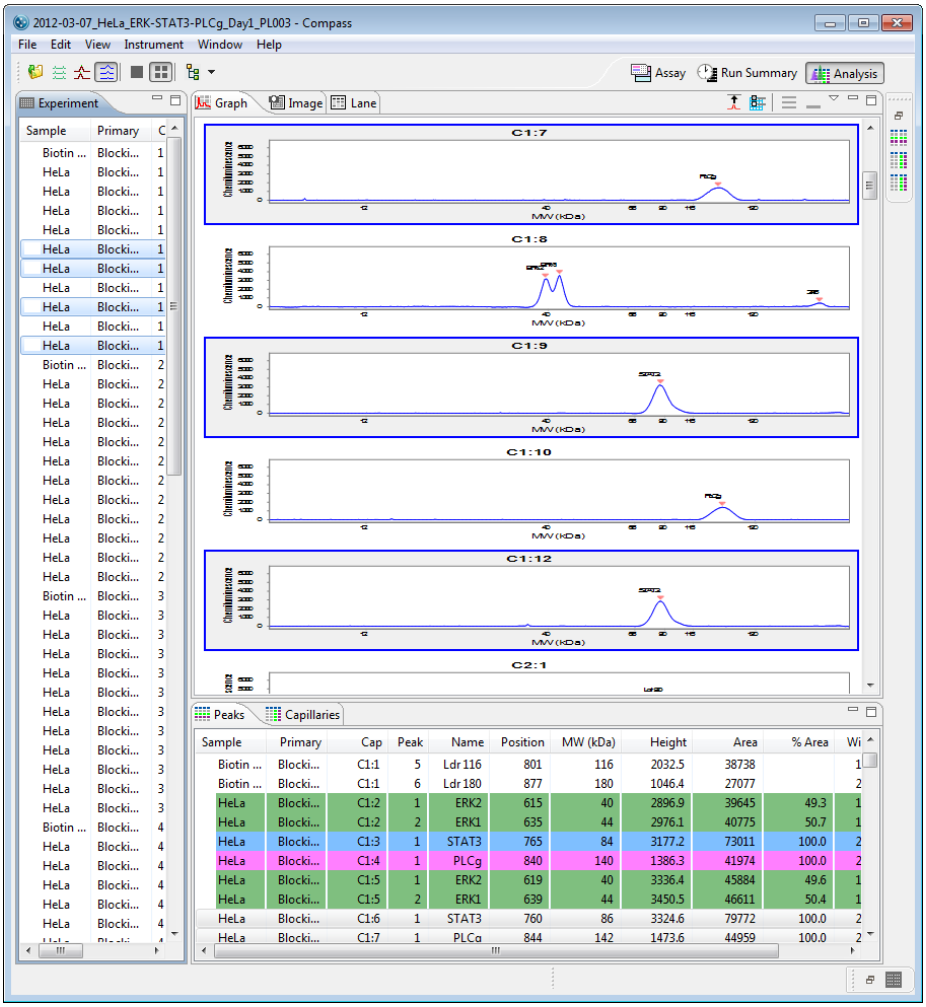
- Electropherograms in the graph pane display overlaid or stacked (depending on the graph option chosen).
- Results for only the selected row(s) will display in the peaks and capillaries tables.
- The selected row(s) are highlighted in the image pane:



- Lanes for only the selected row(s) are displayed in the lane pane:

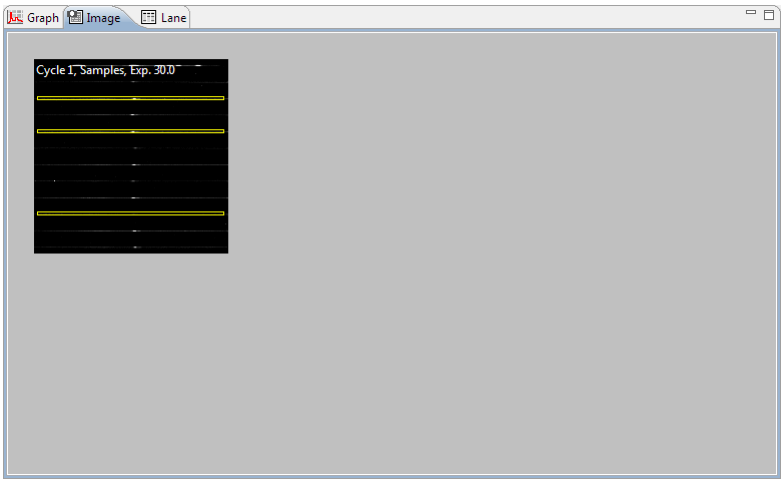


- **To view data up to 96 -capillary format** - Click **Multiple View** in the View bar or select **View** in the main menu and click **Multiple View**:


2012-03-07_HeLa_ERK-STAT3-PLCg_Day1_PL003 - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Experiment
Graph Image Lane
C1:7
C1:8
C1:9
C1:10
C1:12
C2:1
MW (kDa)
Peaks Capillaries
Sample Primary Cap Peak Name Position MW (kDa) Height Area % Area
Biotin ... Blocki... C1:1 5 Ldr 116 801 116 2032.5 38738
Biotin ... Blocki... C1:1 6 Ldr 180 877 180 1046.4 27077
HeLa Blocki... C1:2 1 ERK2 615 40 2896.9 39645 49.3
HeLa Blocki... C1:2 2 ERK1 635 44 2976.1 40775 50.7
HeLa Blocki... C1:3 1 STAT3 765 84 3177.2 73011 100.0
HeLa Blocki... C1:4 1 PLCg 840 140 1386.3 41974 100.0
HeLa Blocki... C1:5 1 ERK2 619 40 3336.4 45884 49.6
HeLa Blocki... C1:5 2 ERK1 639 44 3450.5 46611 50.4
HeLa Blocki... C1:6 1 STAT3 760 86 3324.6 79772 100.0
HeLa Blocki... C1:7 1 PLCg 844 142 1473.6 44959 100.0

Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms corresponding to the selected row(s) are highlighted and displayed in the graph pane.
- Results for selected capillaries display in the peaks and capillaries tables and are highlighted.
- The selected row(s) are highlighted in the image pane:



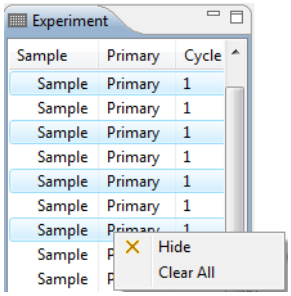
- All selected lanes display in the lane pane, and lanes corresponding to the selected row(s) are highlighted.

Hiding Capillary Data

Capillary data can be hidden from view if needed. To do this:

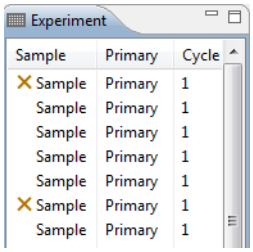
1. Click the **Experiment** tab.

2. Select the rows you want to hide, then right click on one of the selected rows and click **Hide**:



Data for selected rows will be hidden in all data views and results tables (except for the image pane).

- **To view hidden rows** - Select **View** in the main menu and click **Show Hidden**. Hidden rows will become visible again in all panes, and hidden rows will be marked with an X in the experiment pane:



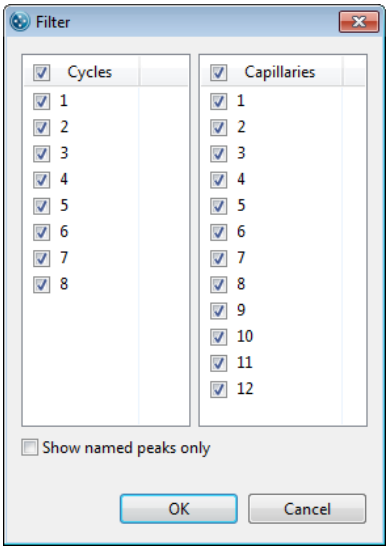
- **To unhide rows** - Select the hidden row(s). Right click on one of the selected rows and click **Unhide.**

Setting Run Data Display Filters

The filter lets you auto-hide specific capillaries in the run file data, or only show data for peaks that Compass identified automatically using the user-defined peak name analysis parameters.

NOTE: When more than one run file is open, filter settings will be applied to all files.

- **To filter data to show specific capillaries only** - Select **View** in the main menu and click **Filter**. Uncheck the boxes for the capillaries you do not want shown, then click **OK**.



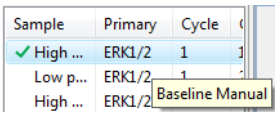
Only data for the checked capillaries will display in data views and results tables, unchecked capillaries will be hidden.

- **To filter data to show named peaks only** - Select **View** in the main menu and click **Filter**. Select **Show named peaks only** then click **OK**. Only data for peaks that Compass identified automatically using the user-defined peak name analysis parameters will display in data views and results tables, all other peak data will be hidden.

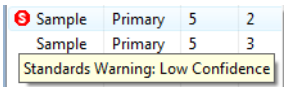
Compass Run Data Notifications and Warnings

If Compass detects a potential data issue, a notification or warning will display next to the row in the experiment pane. A list of warnings, notifications and corrective actions are as follows:

- **Manual correction of sample data notification** - Indicates the user modified sample data manually, such as adding or removing a sample peak. Rolling the mouse over the icon displays the type of data modification made.

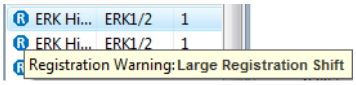


- **Standards warning -** Indicates that one of the standards may not be identified properly. This can be resolved by manually identifying the standards. Please refer to "Step 2 – Checking Fluorescent Sizing Standards" on page 164 for details. Rolling the mouse over the icon displays warning details.



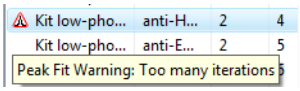
- **Manual correction of standards data notification** - Indicates the user modified the standards data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Registrations warning -** Indicates that a capillary registration was not identified properly. This can be resolved by manually identifying the registration peak. Please refer to "Step 3 – Checking Capillary Registrations" on page 168 for details. Rolling the mouse over the icon displays warning details.



- **Manual correction of registrations notification -** Indicates the user modified the capillary registration data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Peak fit warning -** Indicates that a peak cannot be fit properly. This is typically caused either by a very small peak in the electropherogram or a peak that is very close to the end of the molecular weight range. This can be resolved by removing the peak. Please refer to "Step 4 – Checking the Ladder" on page 169 or "Step 5 – Checking Samples" on page 171 for details. Rolling the mouse over the icon displays warning details.



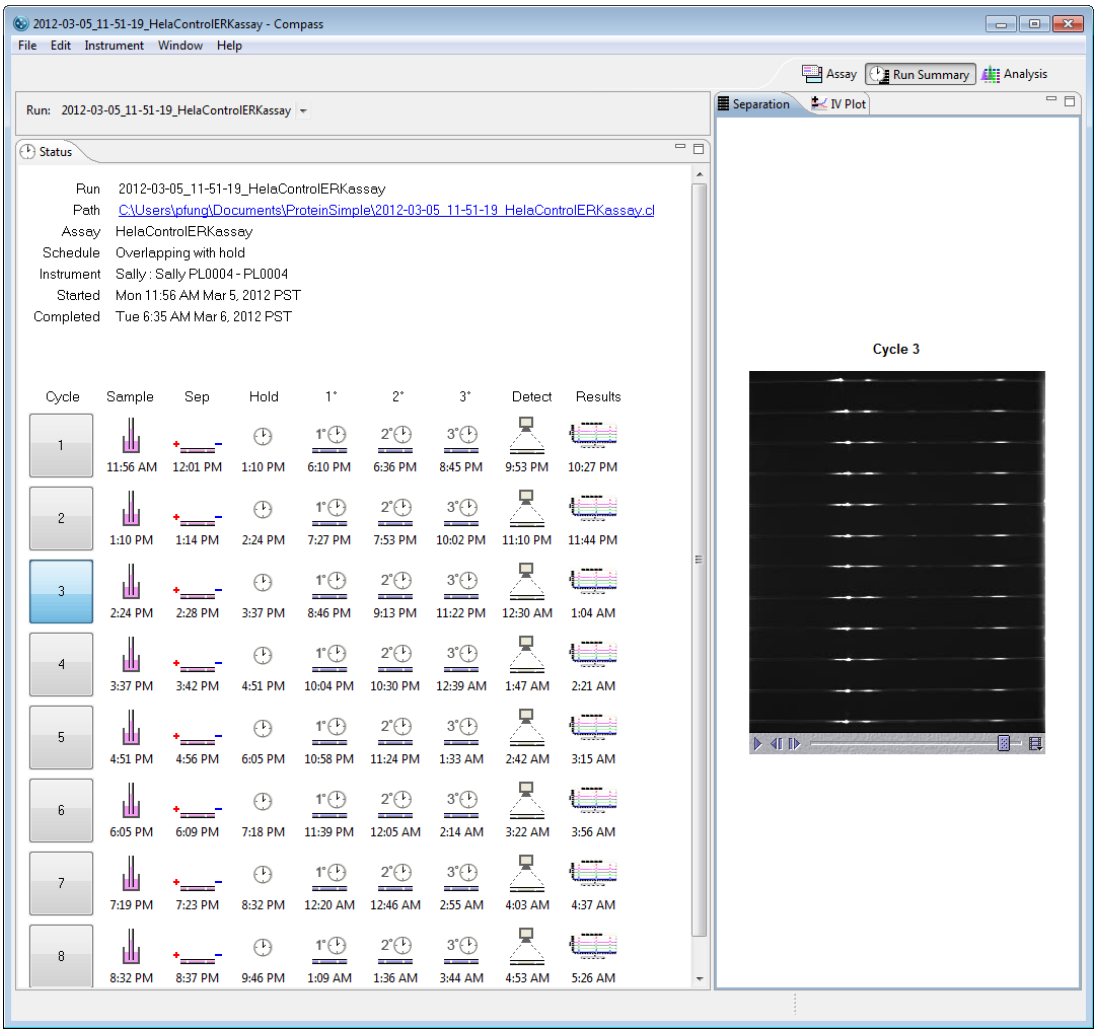
Checking Your Results

Compass detects immunodetected proteins, fluorescent standards and capillary registrations and reports results automatically with no user-interaction. However, ProteinSimple recommends users review their data as outlined in this section as good general practice to ensure accurate results. If a data warning is presented in the experiment pane, the following steps will also help you identify and correct any issues.

Step 1 – Review the Fluorescent Sizing Standards Movie

All capillaries should have three fluorescent sizing standards. To verify the standards separated in the capillary correctly:

1. When the run has completed, click the **Run Summary** screen tab.
2. Click the **Separation** tab and play the movie (this will be the fluorescent standards separation).


2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit Instrument Window Help
Assay Run Summary Analysis
Run: 2012-03-05_11-51-19_HelaControlERKassay
Status
Run 2012-03-05_11-51-19_HelaControlERKassay
Path C:\Users\pfung\Documents\ProteinSimple\2012-03-05_11-51-19_HelaControlERKassay.cl
Assay HelaControlERKassay
Schedule Overlapping with hold
Instrument Sally : Sally PL0004 - PL0004
Started Mon 11:56 AM Mar 5, 2012 PST
Completed Tue 6:35 AM Mar 6, 2012 PST
Cycle Sample Sep Hold 1° 2° 3° Detect Results
1 11:56 AM 12:01 PM 1:10 PM 6:10 PM 6:36 PM 8:45 PM 9:53 PM 10:27 PM
2 1:10 PM 1:14 PM 2:24 PM 7:27 PM 7:53 PM 10:02 PM 11:10 PM 11:44 PM
3 2:24 PM 2:28 PM 3:37 PM 8:46 PM 9:13 PM 11:22 PM 12:30 AM 1:04 AM
4 3:37 PM 3:42 PM 4:51 PM 10:04 PM 10:30 PM 12:39 AM 1:47 AM 2:21 AM
5 4:51 PM 4:56 PM 6:05 PM 10:58 PM 11:24 PM 1:33 AM 2:42 AM 3:15 AM
6 6:05 PM 6:09 PM 7:18 PM 11:39 PM 12:05 AM 2:14 AM 3:22 AM 3:56 AM
7 7:19 PM 7:23 PM 8:32 PM 12:20 AM 12:46 AM 2:55 AM 4:03 AM 4:37 AM
8 8:32 PM 8:37 PM 9:46 PM 1:09 AM 1:36 AM 3:44 AM 4:53 AM 5:26 AM
Separation IV Plot
Cycle 3

3. For each capillary, verify that the standards visibly separated. Each fluorescent point in the capillary represents one of the three sizing standards.

Step 2 – Checking Fluorescent Sizing Standards

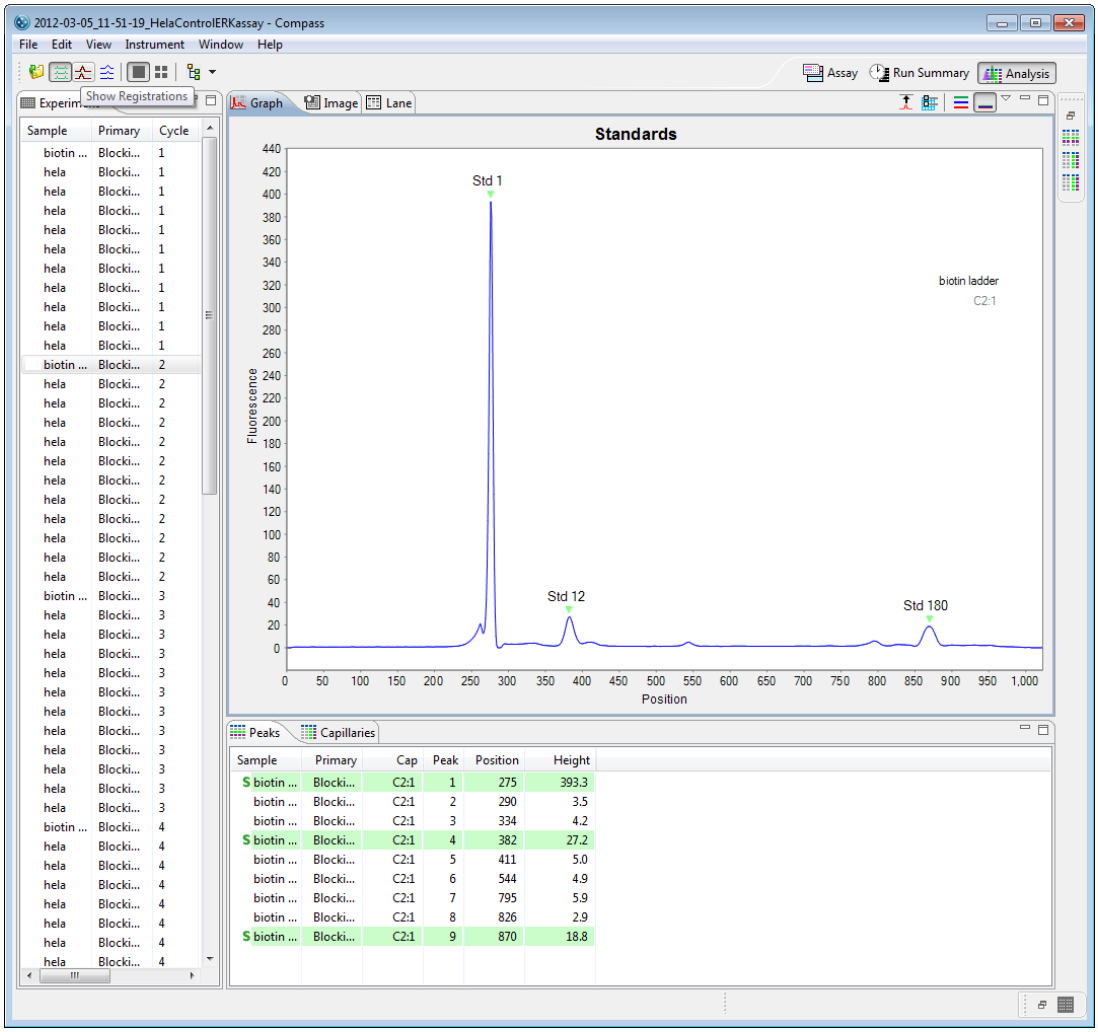
To verify the standards are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Standards** in the View bar. Verification that the standards have been correctly identified can be done in either the graph or lane panes.

Graph Pane:

a. Click **Single View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Graph** tab. Check that the electropherogram has three standard peaks labeled Std 1.0, Std 12.0 and Std 180.0. They will also be identified with a green **S** in the peaks table.


2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Show Registrations
Graph Image Lane
Standards
Std 1
Std 12
Std 180
biotin ladder
C2:1
Fluorescence
Position
Peaks Capillaries
Sample Primary Cycle
Sample Primary Cap Peak Position Height
S biotin ... Blocki... C2:1 1 275 393.3
biotin ... Blocki... C2:1 2 290 3.5
biotin ... Blocki... C2:1 3 334 4.2
S biotin ... Blocki... C2:1 4 382 27.2
biotin ... Blocki... C2:1 5 411 5.0
biotin ... Blocki... C2:1 6 544 4.9
biotin ... Blocki... C2:1 7 795 5.9
biotin ... Blocki... C2:1 8 826 2.9
S biotin ... Blocki... C2:1 9 870 18.8

If standards are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a standard** - Right click the peak in the electropherogram or peaks table and select **Not a Standard**. Compass should correctly reassign the remaining peaks as standards, and update the peaks table.

- **To set an unidentified peak as a standard** - Right click the peak in the electropherogram or peaks table and select **Force Standard**. Compass will assign the peak as a standard, and correctly reassign the remaining standard peaks. A lock icon indicating the standard was set manually will display next to the peak in the peaks table.

---

*NOTE: To remove standards peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Clear**.*

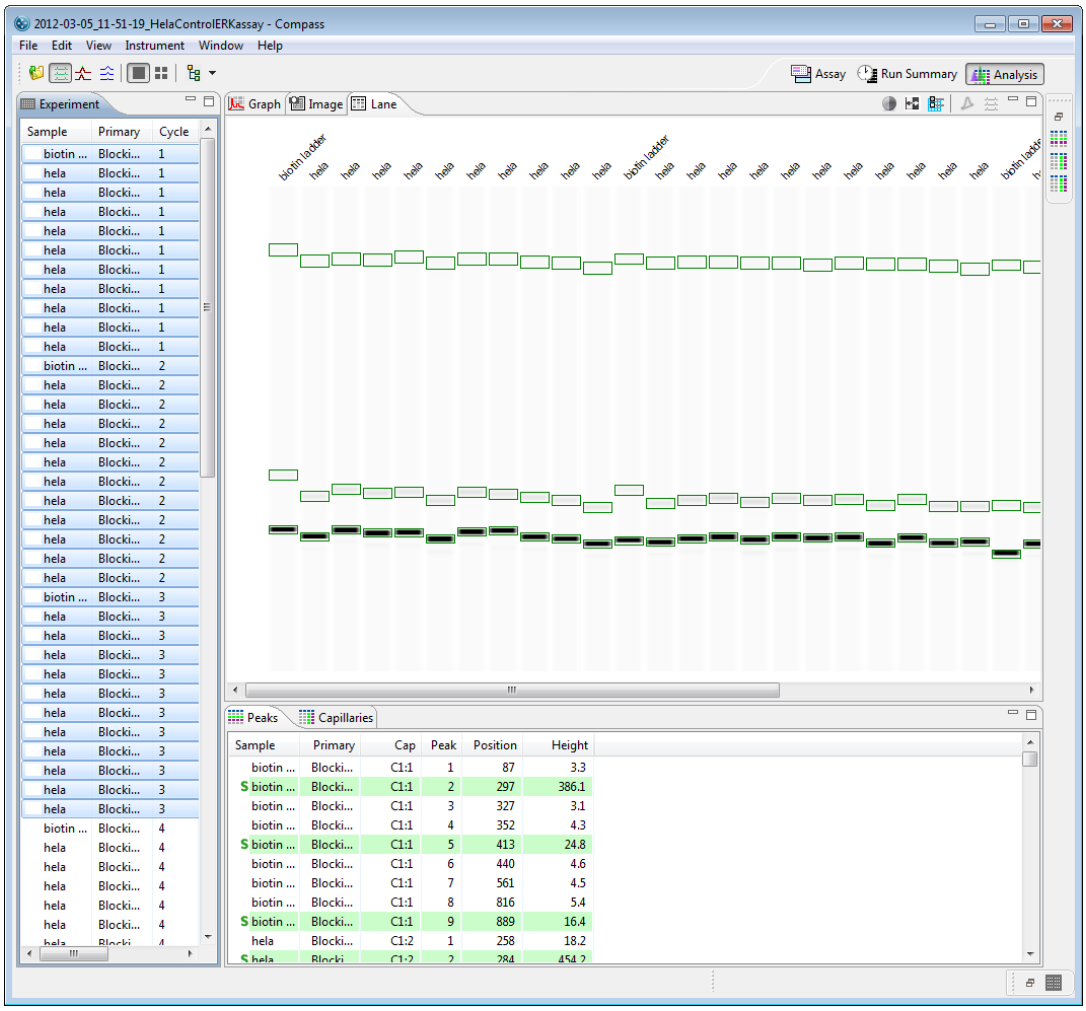
---

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Lane Pane:

a. Click **Multiple View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Lane** tab. Standards will be bands and identified with a green outline. Check that there are three standard bands labeled Std 1.0, Std 12.0 and Std 180.0. They will also be identified with a green **S** in the peaks table. To view band labels, roll the mouse over the individual bands.



If standards are not identified correctly, they can be manually corrected as follows:

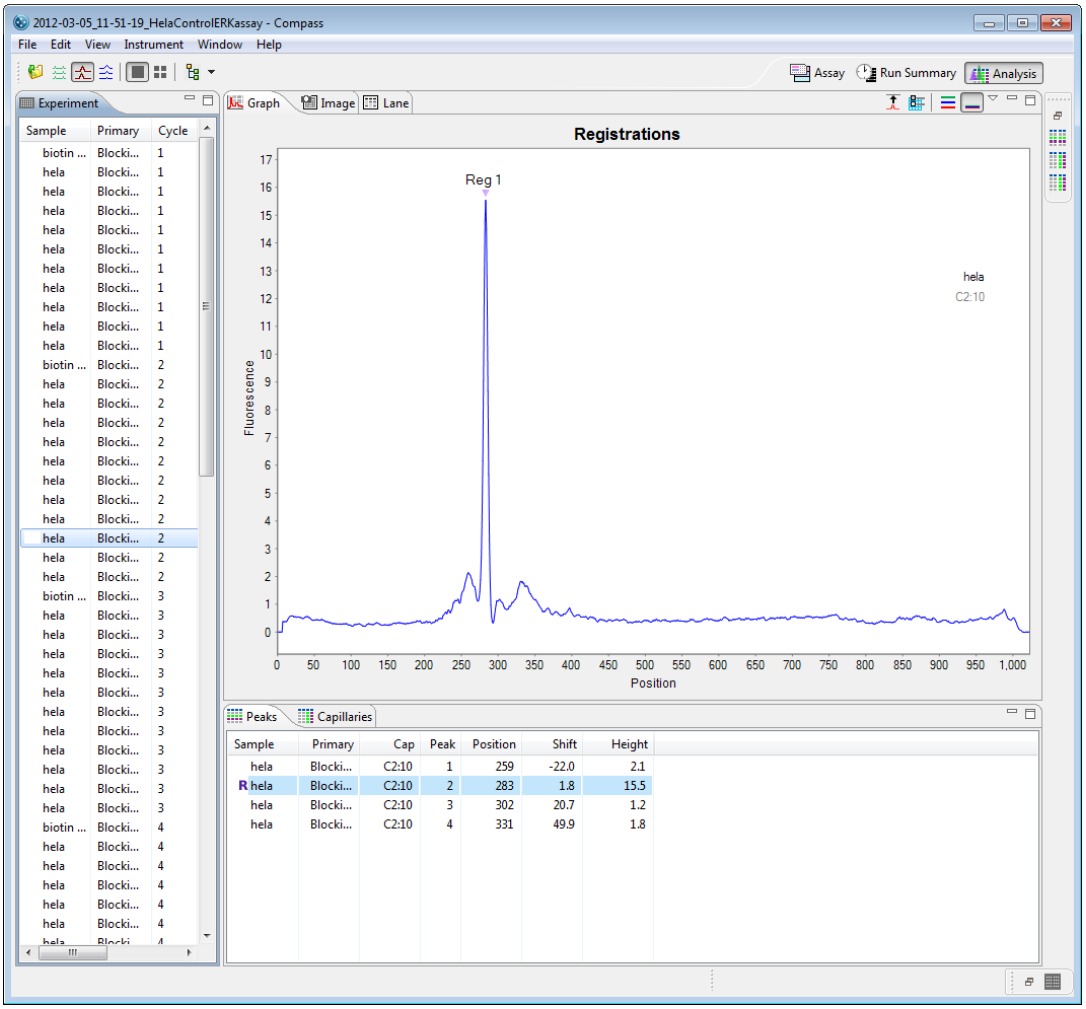
- **If an incorrect band is identified as a standard** - Right click the band in the lane or peaks table and select **Not a Standard**. Compass should correctly reassign the remaining bands as standards.
- **To set an unidentified band as a standard** - Right click the band in the lane or peaks table and select **Force Standard**. Compass will assign the band as a standard, and correctly reassign the remaining standard bands.

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Step 3 – Checking Capillary Registrations

All capillaries should have a single capillary registration peak. To verify the registrations are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Registrations** and **Single View** in the View bar.
3. Click the **Graph** tab.
4. Click on the first row in the experiment pane. The registration peak will be the first and largest peak in the electropherogram. Check that the registration peak is identified and labeled Reg 1 in the electropherogram. It will also be identified with a purple **R** in the peaks table.



5. If the registration peak is not identified correctly, right click the correct registration peak in the electropherogram or peaks table and select **Registration Force**. Compass will assign the new peak as the capillary registration. A lock icon indicating the registration was set manually will display next to the peak in the peaks table.

---

*NOTE: To remove registration peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Registration Clear**.*

---

6. Repeat the previous steps for the remaining rows in the experiment pane to make sure all registrations are identified correctly.

Step 4 – Checking the Ladder

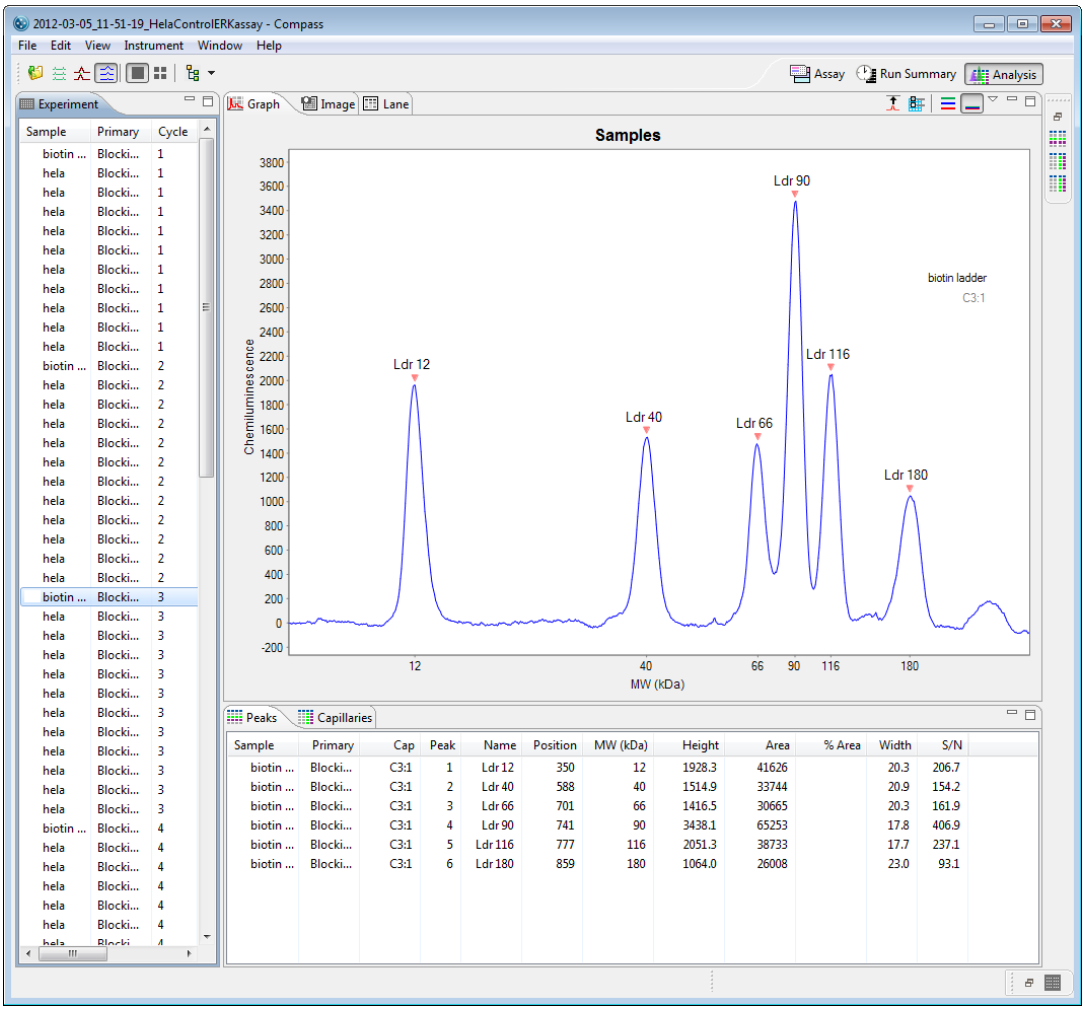
The biotinylated ladder should have six sizing standards at 12, 40, 66, 90, 116 and 180 kDa. To verify the ladder standards are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Samples** in the View bar. Verification that the ladder standards have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

   Graph Pane:

   a. Click **Single View** in the View bar.

   b. Click on the row in the experiment pane that contains the ladder (typically row 1), then click the **Graph** tab. Check that the electropherogram has six ladder peaks labeled Ldr 12, Ldr 40, Ldr 66, Ldr 90, Ldr 116 and Ldr 180.



If ladder peaks are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a ladder peak** - Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass should correctly reassign the remaining peaks as ladder standards.
- **To set an unidentified peak as a ladder peak** - Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass will assign the peak as a ladder standard, and correctly reassign the remaining ladder standards peaks.

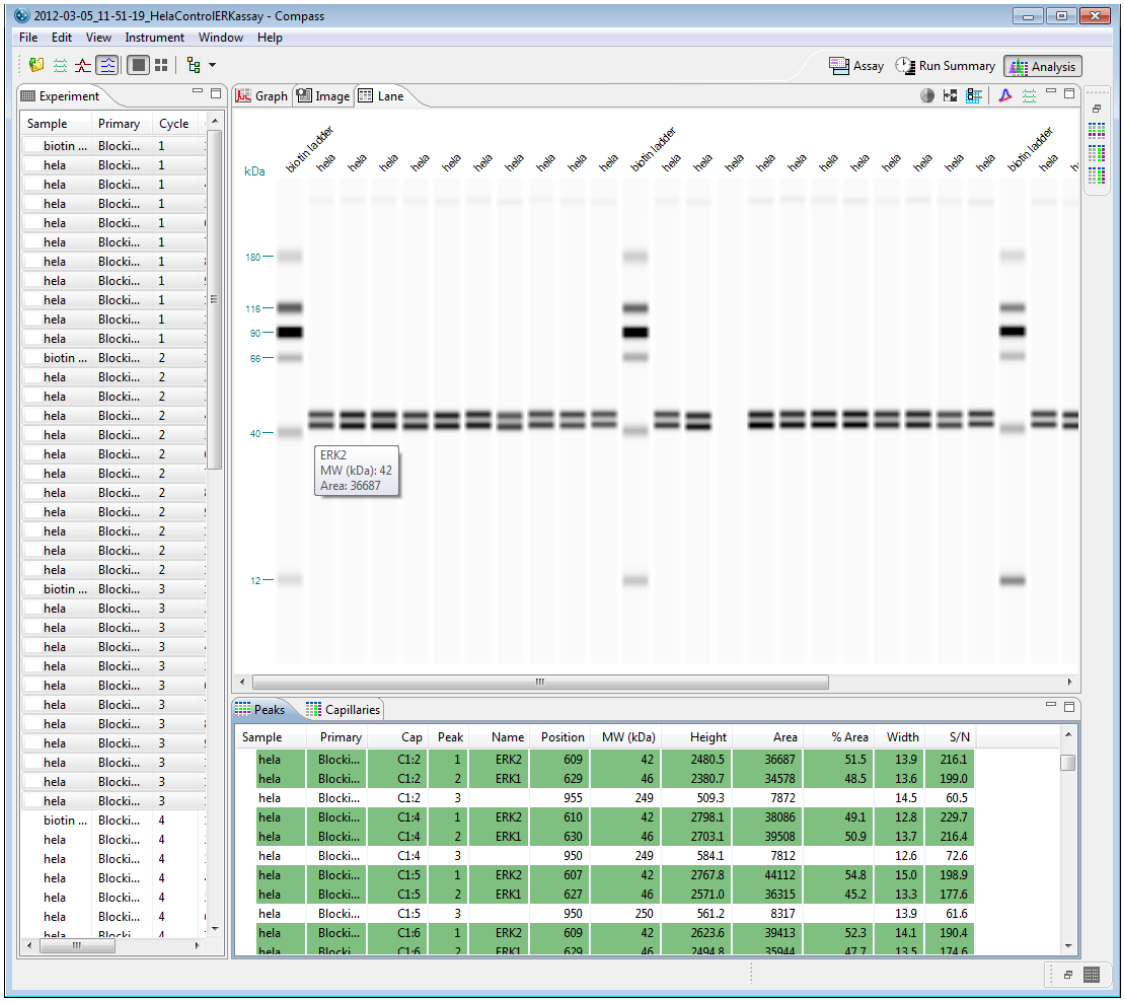
---

*NOTE: To remove ladder peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

Lane Pane:

a. Click either **Single View** or **Multiple View** in the View bar.

b. Click on the row in the experiment pane that contains the ladder (typically row 1), then click the **Lane** tab. Check that the lane has six ladder bands labeled Ldr 12, Ldr 40, Ldr 66, Ldr 90, Ldr 116 and Ldr 180.0. To view band labels, roll the cursor over the individual bands. If ladder bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.


2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Experiment
Graph Image Lane
kDa
180
116
90
66
40
12
ERK2
MW (kDa): 42
Area: 36687
Peaks Capillaries
Sample Primary Cap Peak Name Position MW (kDa) Height Area % Area Width S/N
hela Blocki... C1:2 1 ERK2 609 42 2480.5 36687 51.5 13.9 216.1
hela Blocki... C1:2 2 ERK1 629 46 2380.7 34578 48.5 13.6 199.0
hela Blocki... C1:2 3 955 249 509.3 7872 14.5 60.5
hela Blocki... C1:4 1 ERK2 610 42 2798.1 38086 49.1 12.8 229.7
hela Blocki... C1:4 2 ERK1 630 46 2703.1 39508 50.9 13.7 216.4
hela Blocki... C1:4 3 950 249 584.1 7812 12.6 72.6
hela Blocki... C1:5 1 ERK2 607 42 2767.8 44112 54.8 15.0 198.9
hela Blocki... C1:5 2 ERK1 627 46 2571.0 36315 45.2 13.3 177.6
hela Blocki... C1:5 3 950 250 561.2 8317 13.9 61.6
hela Blocki... C1:6 1 ERK2 609 42 2623.6 39413 52.3 14.1 190.4

Step 5 – Checking Samples

All immunodetected sample proteins in the graph and lane panes will be labeled automatically with the calculated protein size.

NOTE: The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

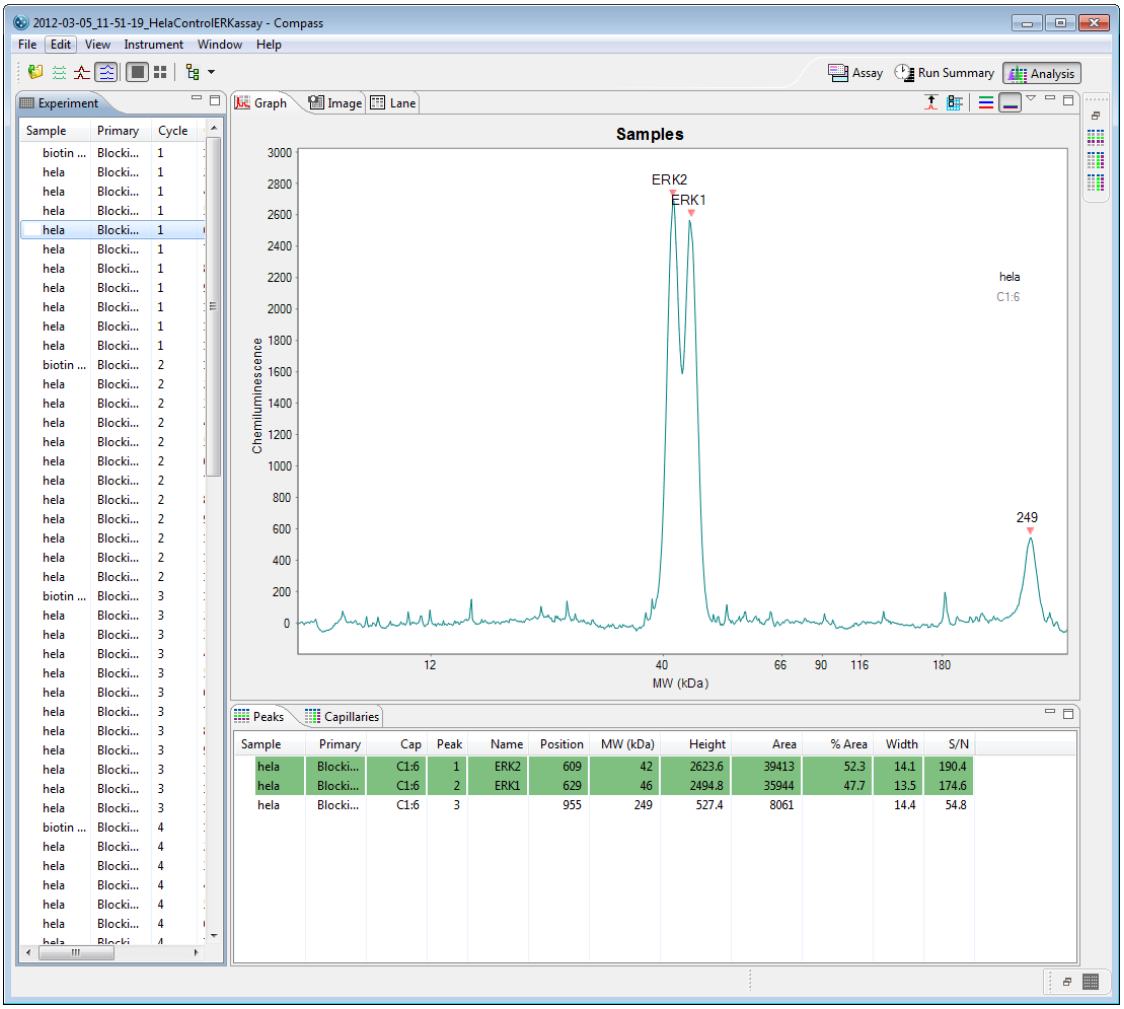
To verify that sample proteins are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Samples** in the View bar. Verification that sample proteins have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

   Graph Pane:

   a. Click **Single View** in the View bar.

   b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Graph** tab.



If sample peaks are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.
- **To set an unidentified peak as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

---

*NOTE: To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

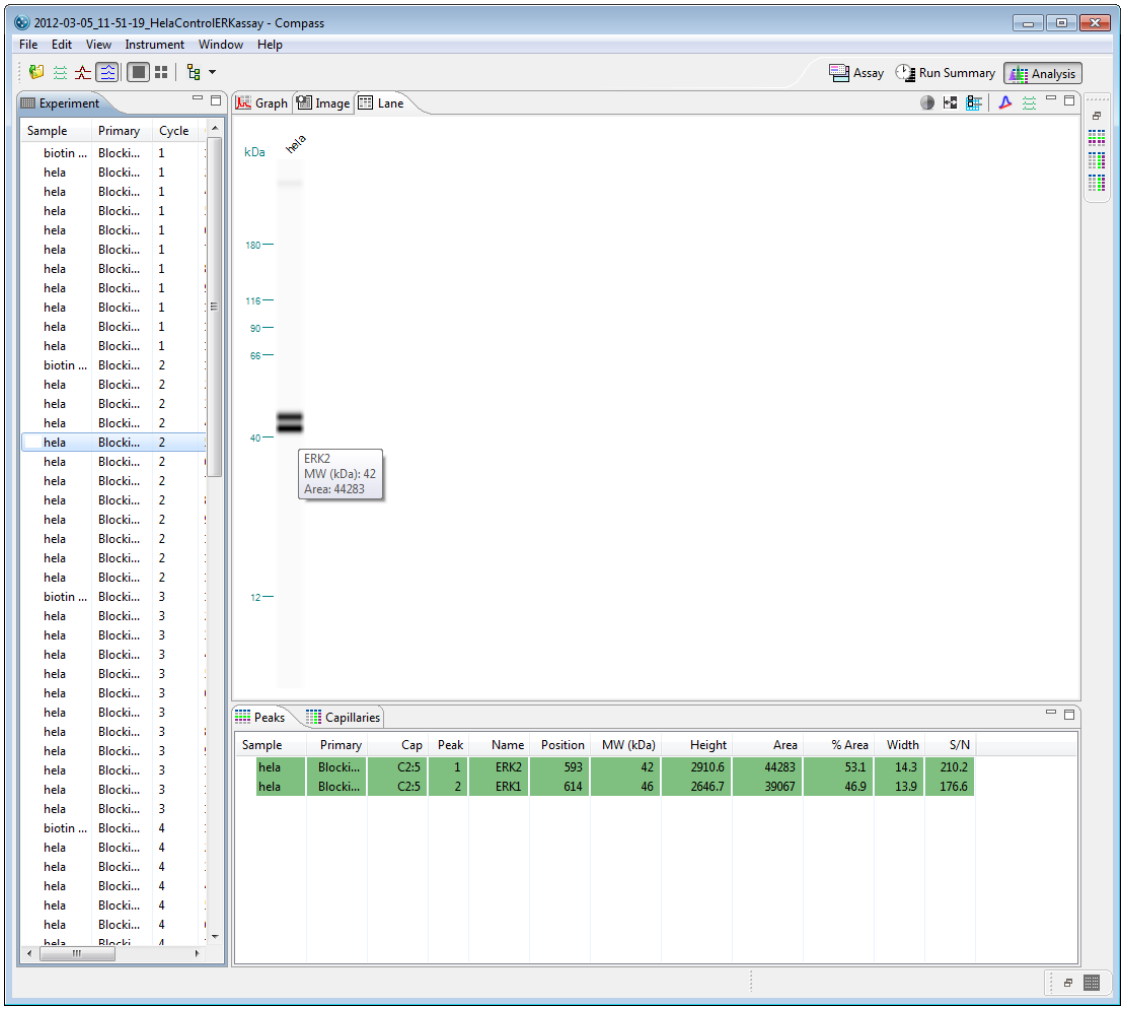
---

c. Repeat the previous steps for the remaining sample rows in the experiment pane to make sure all sample proteins are identified correctly.

Lane Pane:

a. Click either **Single View** or **Multiple View** in the View bar.

b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Lane** tab. To view sample protein band labels, roll the cursor over the individual bands. If sample bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.



Step 6 – Assigning Peak Names (Optional)

Compass can identify and automatically name sample proteins associated with specific primary antibodies in the run data using user-specified peak name settings. For more information on how to do this see "Peak Names Settings" on page 238.

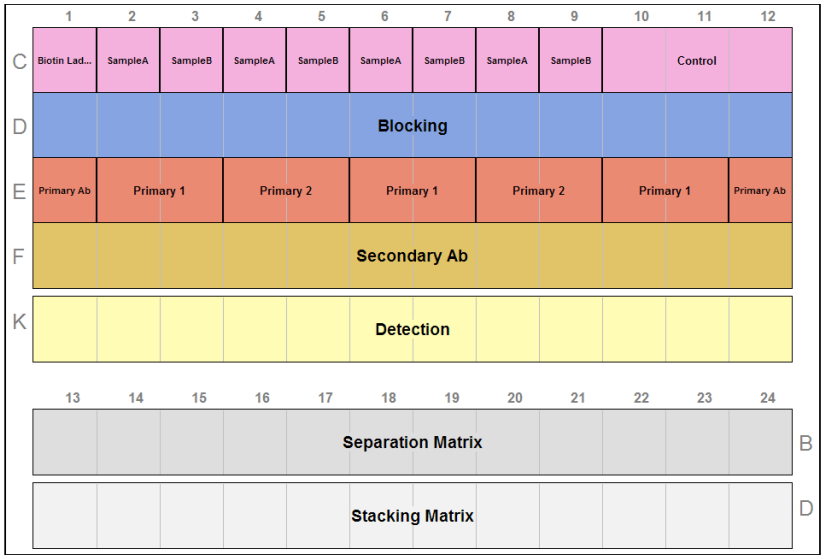
Group Statistics

The Grouping View is used to analyze replicates by calculating the mean, standard deviation and CV of named proteins (see "Peak Names Settings" on page 238 for detailed information on entering named proteins). Statistics for each protein are also plotted for easy comparison between samples, antibodies, and proteins.

Using Groups

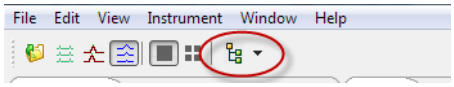
1. A group is automatically created for capillaries with the same sample and primary antibody name, so to use this feature, this information must first be entered into the Assay template. To do this:
   a. Click the **Assay** tab and go to the Template pane.
   b. Enter sample names and primary antibody names as described in "Step 6 - Add Assay Plate Annotations (Optional)" on page 36. Be sure to enter the same sample and/or primary antibody names for the groups of samples you want to calculate statistics for.

      In this example there are two sample types, **Sample A** and **Sample B** which were run with two different antibodies, **Primary 1** and **Primary 2**.

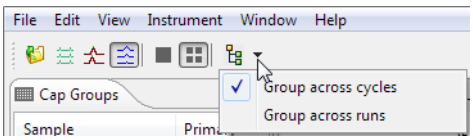


Each of the two samples were run with each of the two antibodies twice in every cycle. This creates four groups for the combination of two samples and two antibodies.

2. Select the **Analysis** tab and click the **Grouping View** icon in the view bar. Clicking this icon toggles the grouping view on and off.



3. Select a grouping option by clicking the arrow next to the grouping view icon. These options allow you to group capillaries in multiple ways:

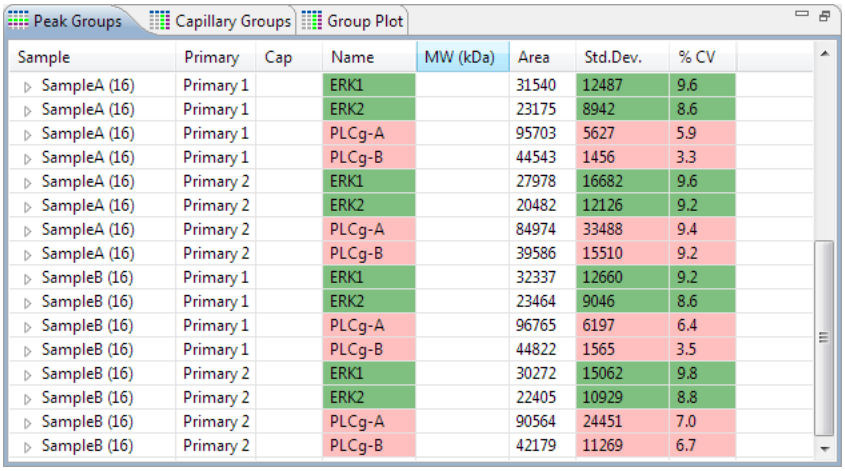


- **Group across cycles** - Groups capillaries run in different cycles.
- **Group across runs** - Groups capillaries from multiple runs. This option creates fewer groups and generates statistics across multiple runs.
- **No option selected** - When only one run is open, groups will only contain capillaries from the same cycle. When more than one run is open, groups are created for each run.

Viewing Statistics

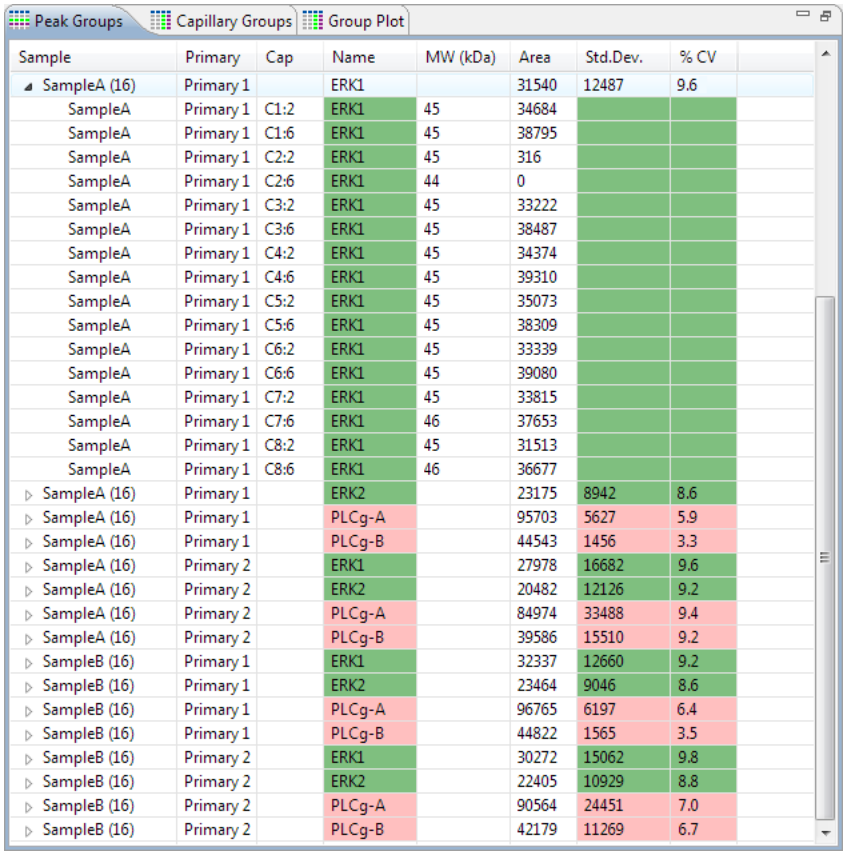
Peak and Capillary Groups

The Peak Groups pane reports statistics for each named protein in every group. Each group shows the statistics for named proteins which includes average area, standard deviation and %CV. The number in parenthesis after the sample name indicates the number of capillaries in the group.



Sample	Primary	Cap	Name	MW (kDa)	Area	Std.Dev.	% CV
SampleA (16)	Primary 1		ERK1		31540	12487	9.6
SampleA (16)	Primary 1		ERK2		23175	8942	8.6
SampleA (16)	Primary 1		PLCg-A		95703	5627	5.9
SampleA (16)	Primary 1		PLCg-B		44543	1456	3.3
SampleA (16)	Primary 2		ERK1		27978	16682	9.6
SampleA (16)	Primary 2		ERK2		20482	12126	9.2
SampleA (16)	Primary 2		PLCg-A		84974	33488	9.4
SampleA (16)	Primary 2		PLCg-B		39586	15510	9.2
SampleB (16)	Primary 1		ERK1		32337	12660	9.2
SampleB (16)	Primary 1		ERK2		23464	9046	8.6
SampleB (16)	Primary 1		PLCg-A		96765	6197	6.4
SampleB (16)	Primary 1		PLCg-B		44822	1565	3.5
SampleB (16)	Primary 2		ERK1		30272	15062	9.8
SampleB (16)	Primary 2		ERK2		22405	10929	8.8
SampleB (16)	Primary 2		PLCg-A		90564	24451	7.0
SampleB (16)	Primary 2		PLCg-B		42179	11269	6.7

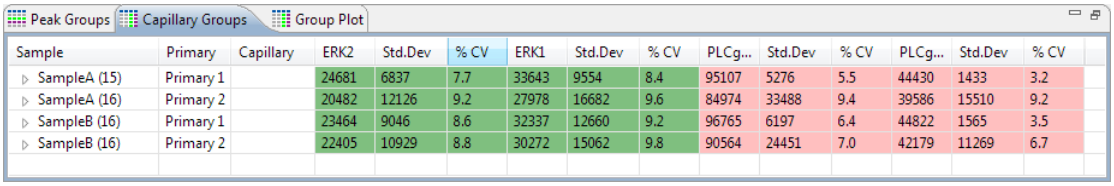
Clicking the arrow next to a group lists the individual capillaries in the group and reported data for each capillary:



Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Cap	Name	MW (kDa)	Area	Std.Dev.	% CV
SampleA (16)	Primary 1		ERK1		31540	12487	9.6
SampleA	Primary 1	C1:2	ERK1	45	34684		
SampleA	Primary 1	C1:6	ERK1	45	38795		
SampleA	Primary 1	C2:2	ERK1	45	316		
SampleA	Primary 1	C2:6	ERK1	44	0		
SampleA	Primary 1	C3:2	ERK1	45	33222		
SampleA	Primary 1	C3:6	ERK1	45	38487		
SampleA	Primary 1	C4:2	ERK1	45	34374		
SampleA	Primary 1	C4:6	ERK1	45	39310		
SampleA	Primary 1	C5:2	ERK1	45	35073		
SampleA	Primary 1	C5:6	ERK1	45	38309		
SampleA	Primary 1	C6:2	ERK1	45	33339		
SampleA	Primary 1	C6:6	ERK1	45	39080		
SampleA	Primary 1	C7:2	ERK1	45	33815		
SampleA	Primary 1	C7:6	ERK1	46	37653		
SampleA	Primary 1	C8:2	ERK1	45	31513		
SampleA	Primary 1	C8:6	ERK1	46	36677		
SampleA (16)	Primary 1		ERK2		23175	8942	8.6
SampleA (16)	Primary 1		PLCg-A		95703	5627	5.9
SampleA (16)	Primary 1		PLCg-B		44543	1456	3.3
SampleA (16)	Primary 2		ERK1		27978	16682	9.6
SampleA (16)	Primary 2		ERK2		20482	12126	9.2
SampleA (16)	Primary 2		PLCg-A		84974	33488	9.4
SampleA (16)	Primary 2		PLCg-B		39586	15510	9.2
SampleB (16)	Primary 1		ERK1		32337	12660	9.2
SampleB (16)	Primary 1		ERK2		23464	9046	8.6
SampleB (16)	Primary 1		PLCg-A		96765	6197	6.4
SampleB (16)	Primary 1		PLCg-B		44822	1565	3.5
SampleB (16)	Primary 2		ERK1		30272	15062	9.8
SampleB (16)	Primary 2		ERK2		22405	10929	8.8
SampleB (16)	Primary 2		PLCg-A		90564	24451	7.0
SampleB (16)	Primary 2		PLCg-B		42179	11269	6.7

The **Capillary Groups** pane pivots the **Peak Groups** results to show statistics for named protein peaks in individual columns.

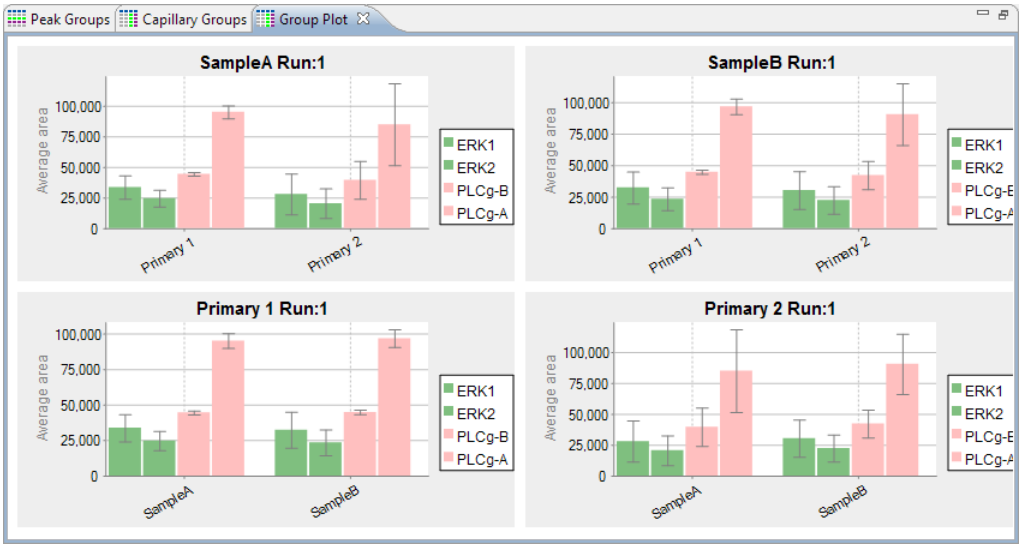


Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Capillary	ERK2	Std.Dev	% CV	ERK1	Std.Dev	% CV	PLCg...	Std.Dev	% CV	PLCg...	Std.Dev	% CV
SampleA (15)	Primary 1		24681	6837	7.7	33643	9554	8.4	95107	5276	5.5	44430	1433	3.2
SampleA (16)	Primary 2		20482	12126	9.2	27978	16682	9.6	84974	33488	9.4	39586	15510	9.2
SampleB (16)	Primary 1		23464	9046	8.6	32337	12660	9.2	96765	6197	6.4	44822	1565	3.5
SampleB (16)	Primary 2		22405	10929	8.8	30272	15062	9.8	90564	24451	7.0	42179	11269	6.7

Group Plots

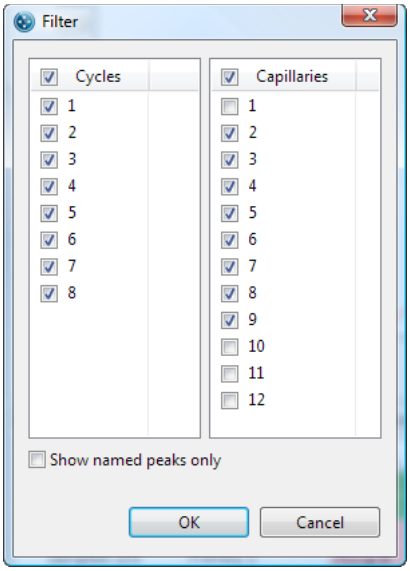
The mean values for named peaks in each group are plotted in bar graphs with error bars showing the standard deviation. The plots compare different antibodies for the same sample and different samples for the same antibody to allow a choice of presentation.



Hiding or Removing Capillaries in Group Analysis

Hidden capillaries are not included in groups. However, hiding capillaries provides an easy way to reject individual capillaries from the statistical analysis. See "Hiding Capillary Data" on page 159 for details on how to do this.

Larger groups of capillaries can also be excluded from analysis. To do this, select **View** and click **Filter**.



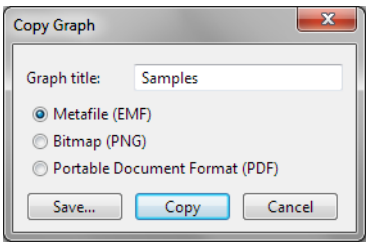
Uncheck the box next to the cycles or capillaries you wish to remove and click **OK**. This data will now be removed from the grouped view statistics.

Copying Data Views and Results Tables

You can copy and paste data and results tables into other documents, or save a data view as a graphic file.

Copying Data Views

1. Click in the graph or lane pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. If you selected copy from the graph pane the following window will display, click **Copy**. If you selected copy from the lane pane skip to the next step.



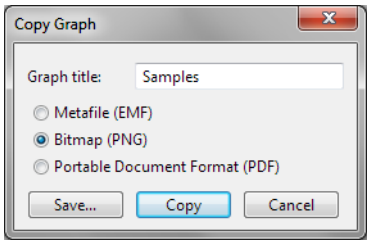
4. Open a document (Word, Excel, PowerPoint, etc). Right click in the document and select **Paste**. A graphic of the copied data view will be pasted into the document.

Copying Results Tables

1. Click in the peaks or capillaries pane.
2. Select one or multiple rows.
3. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
4. Open a document (Word, Excel, PowerPoint, etc). Right click in the document and select **Paste**. Data for the rows selected will be pasted into the document.

Saving the Graph View as an Image File

1. Click in the graph pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. Select an image option (EMF, PNG or PDF) in the pop-up window, then click **Save**.



4. Select a directory to save the file to and enter a file name, then click **OK**.

Exporting Run Files

Results tables and raw plot data can be exported for use in other applications.

Exporting Results Tables

To export the information in the peaks and capillaries tables:

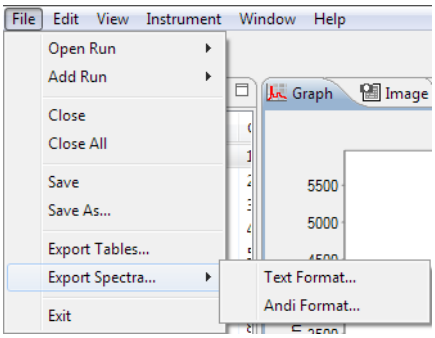
1. Click **File** in the main menu and click **Export Tables**.
2. Select a directory to save the files to and click **OK**. Data will be exported in .txt format.

NOTE: To exclude export of standards data or export results table data in .csv format, see "Setting Data Export Options" on page 391.

Exporting Raw Sample Electropherogram Data

To export raw sample plot data:

1. Click **File** in the main menu and click **Export Spectra**.



- **To export data in .txt format** - Select **Text Format**. Plots will be exported in one file for all capillaries.
- **To export data in .cdf format** - Select **Andi Format**. Plots will be exported in one file per capillary.

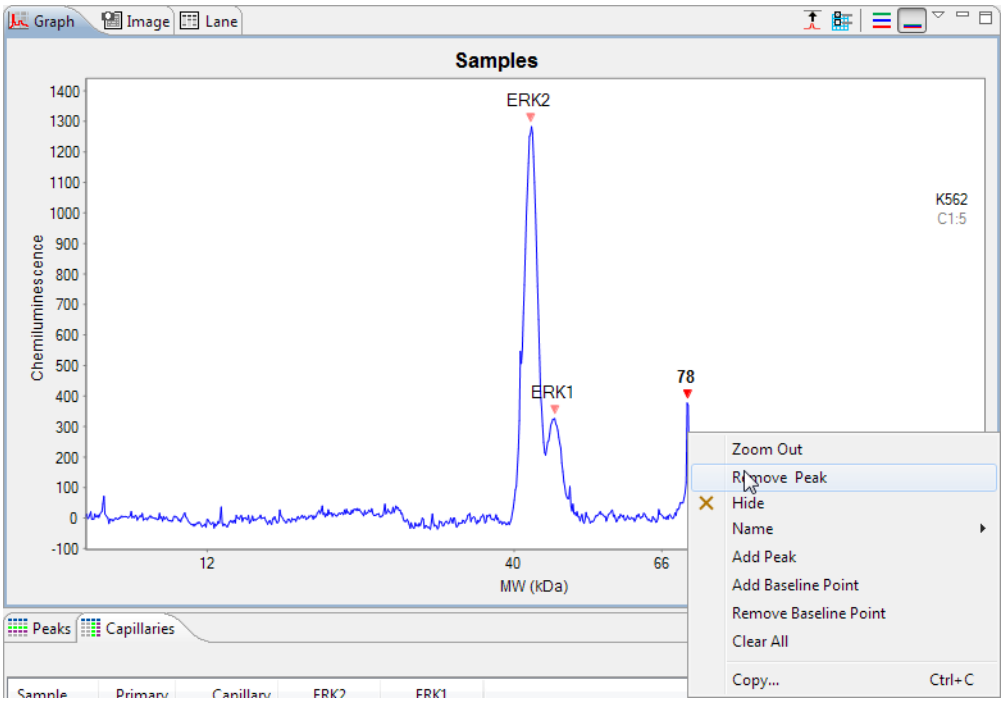
2. Select a directory to save the files to and click **OK**. Data will be exported in the selected format.

Changing Sample Protein Identification

Compass allows you to customize what sample proteins are reported in the results tables by making manual adjustments in the electropherogram or peaks table.

Adding or Removing Sample Data

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
    - **To remove a peak from the data -** Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.



- **To add an unidentified peak to the data -** Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

NOTES:

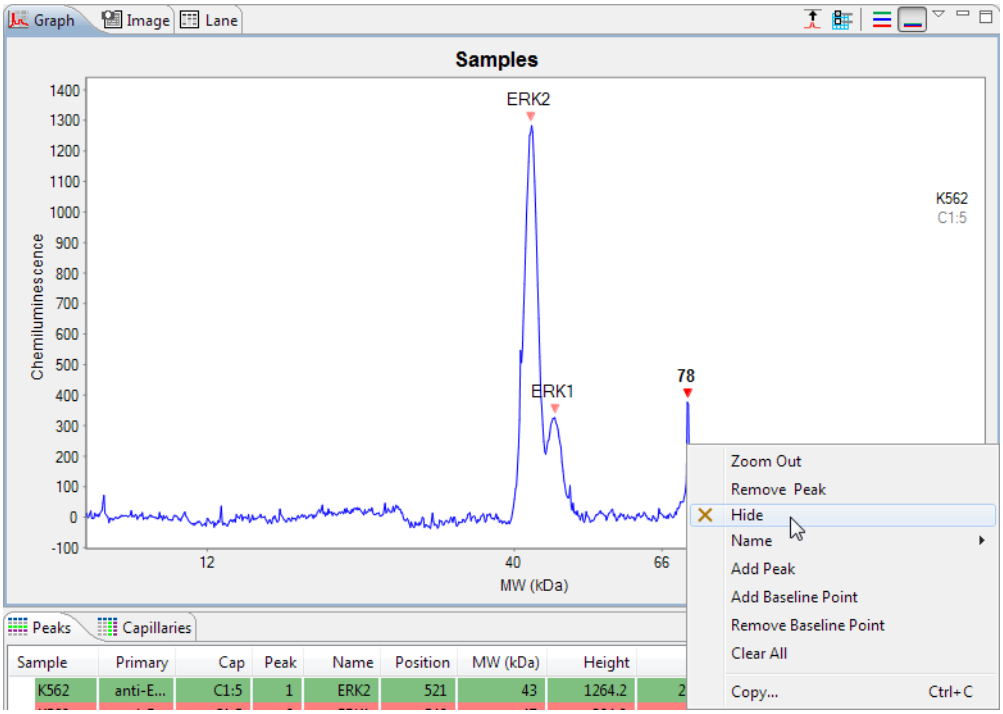
*To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

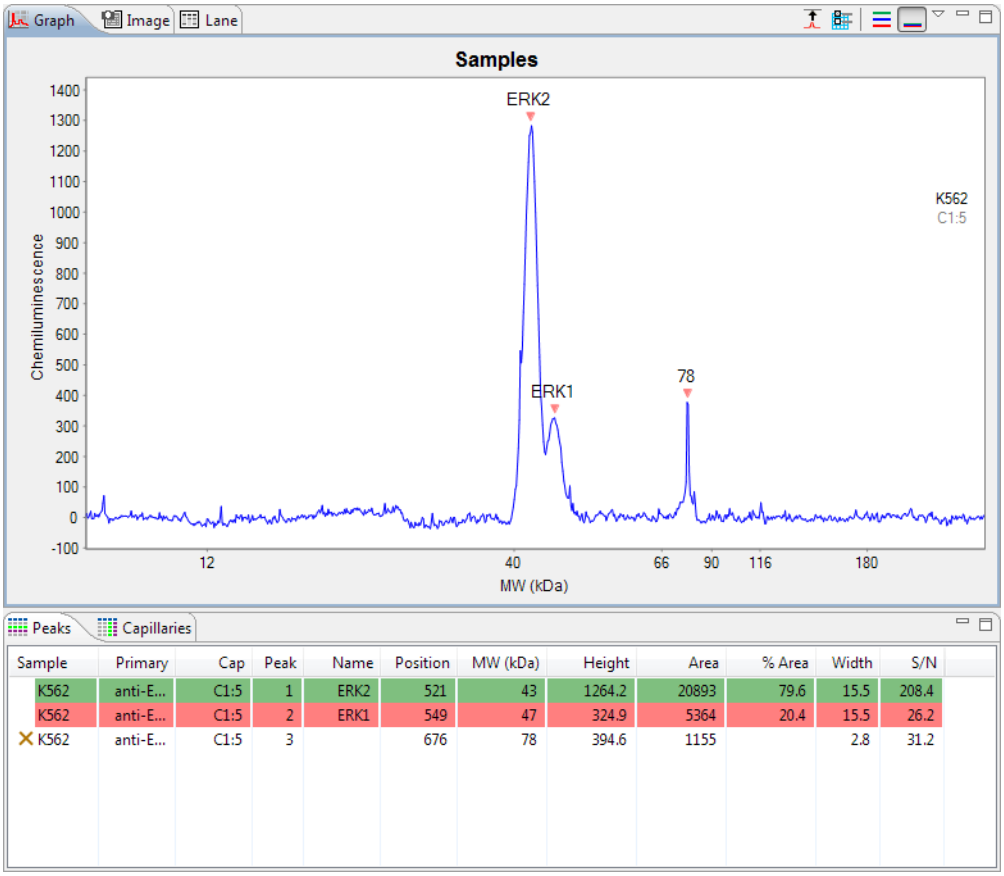
Hiding Sample Data

You can hide the results for a sample protein in the results tables without completely removing it from the reported results. To to this:

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
4. Right click the peak in the electropherogram or peaks table and select **Hide peak**. Compass will hide the peak data in the results tables.



5. To view hidden peak data, click **View** in the main menu and click **Show Hidden**. Hidden peak data will display in the results table and be marked with an **X**.



Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N
K562	anti-E...	C1:5	1	ERK2	521	43	1264.2	20893	79.6	15.5	208.4
K562	anti-E...	C1:5	2	ERK1	549	47	324.9	5364	20.4	15.5	26.2
K562	anti-E...	C1:5	3		676	78	394.6	1155		2.8	31.2

6. To unhide a peak, right click on the peak in the electropherogram or peaks table and select **Unhide Peak**.

Changing Peak Names for Sample Data

If Compass did not automatically name a sample protein associated with a specific primary antibody, this can be adjusted manually. To do this:

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.

4. Right click the peak in the electropherogram or peaks table and click **Name**, then click a name in the list. Compass will change the peak name in the electropherogram and results tables, and adjust peak names for other sample proteins accordingly.

NOTES:

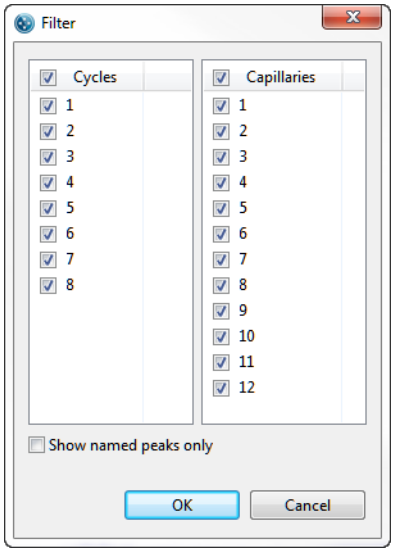
For details on how to specify peak name settings, see "Peak Names Settings" on page 238.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

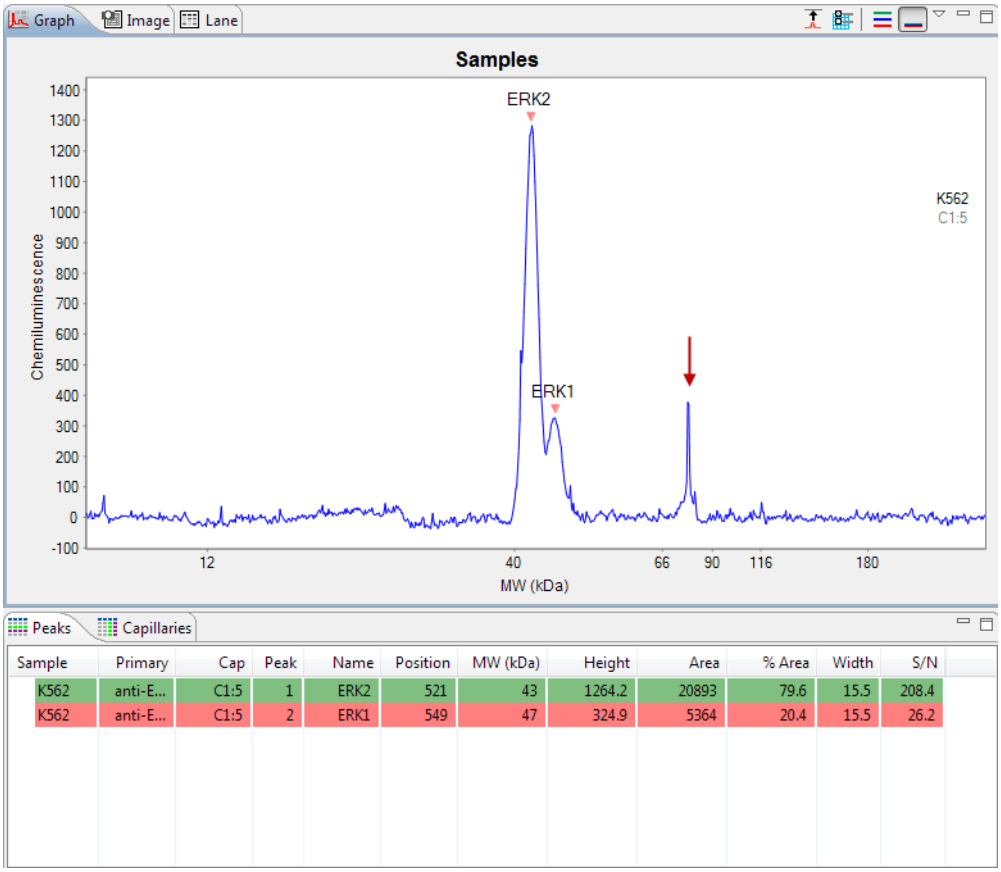
Displaying Sample Data for Named Peaks Only

You can adjust the sample data to display results only for user-specified named peaks. To do this:

1. Click **Show Samples** in the View bar.
2. Click **View** in the main menu and click **Filter**.
3. Check the **Show Named Peaks only** box and click **OK**.



Compass will hide peak data in the electropherogram and results tables for all unnamed peaks, and instead only display data for named peaks in the electropherogram and results tables.



Changing the Virtual Blot View

Options in the lane pane let users change the contrast or invert the virtual blot, remove baseline noise, change lane labels or overlay standards data on sample lanes.

The lane pane toolbar has the following options:

- Contrast Adjustment
- Invert
- Edit Labels
- Remove Baseline
- Overlay Standards Data

Adjusting the Contrast

1. Click the **Contrast Adjustment** button. The contrast tool will display:

2. Click the bar and drag it up or down to adjust the contrast.
3. When finished, click **X** to close the tool.

Inverting the Virtual Blot

1. Click the **Invert** button. The virtual blot image will invert:

2. Click the **Invert** button again to return to the default view.

Selecting Lane Labels

The labels shown above the lanes in the virtual blot can be customized. To do this:

1. Click the **Edit Labels** button. The label box will display:

2. Check one or multiple label boxes, and uncheck those you don't want to display. To remove labels completely, uncheck all boxes. The following label options are available:

   - **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
   - **Blocking** - Blocking reagent name. If a name was entered in the assay template (Assay screen), that name will display here. Otherwise, Blocking (default name) will display.
   - **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
   - **Secondary Ab** - Secondary antibody name. If a secondary antibody name was entered in the assay template (Assay screen), those names will display here. Otherwise, Secondary (default name) will display.
   - **Capillary** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3

NOTE: Peggy runs up to eight cycles (12 capillaries at a time), so the cycle number displayed will always be C1-C8 depending on the number of cycles programmed.

   - **Attributes** - Attribute text. If attribute information was entered for any of the rows or wells in the assay template (Assay screen), up to four can be selected and displayed as lane labels.

Viewing the Uncorrected Sample Baseline

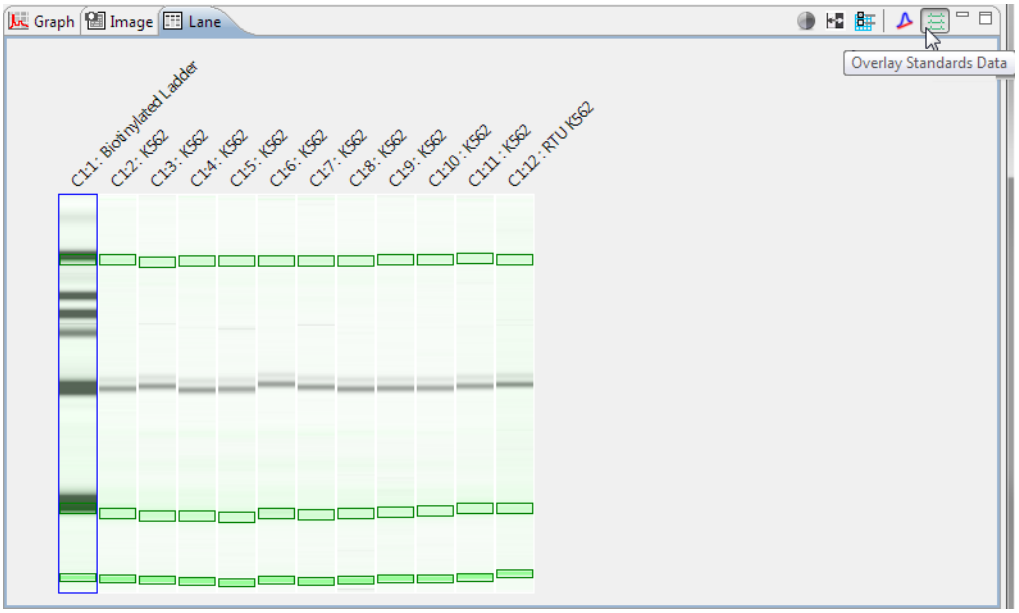
1. Click the **Remove Baseline** button (active for sample data only). This will remove the automatic baseline correction.

2. Click the **Remove Baseline** button again to return to the default, baseline corrected view.

Overlaying Standards Data on Sample Lanes

Data for the standards can be overlaid on the sample data in the virtual blot so users can view the raw, unaligned (uncorrected) lane data. To do this:

1. Click the **Overlay Standards Data** button (active for sample data only). An overlay of the raw sample and standards data will display:



2. Click the **Overlay Standards Data** button again to return to the default, auto-aligned view.

Moving Lanes in the Virtual Blot View

Lanes in the virtual blot view can be moved to make it easier to compare specific lanes of data. To do this:

1. Click a lane in the virtual blot. Hold the mouse button down and drag the lane to a new position.

2. Release the mouse button. The lane will now be repositioned in the virtual blot view.

Changing the Electropherogram View

Options in the graph pane let users zoom and scale electropherograms, overlay or stack plots and change the peak and plot information displayed.

The graph pane toolbar has the following options:

Auto Scale

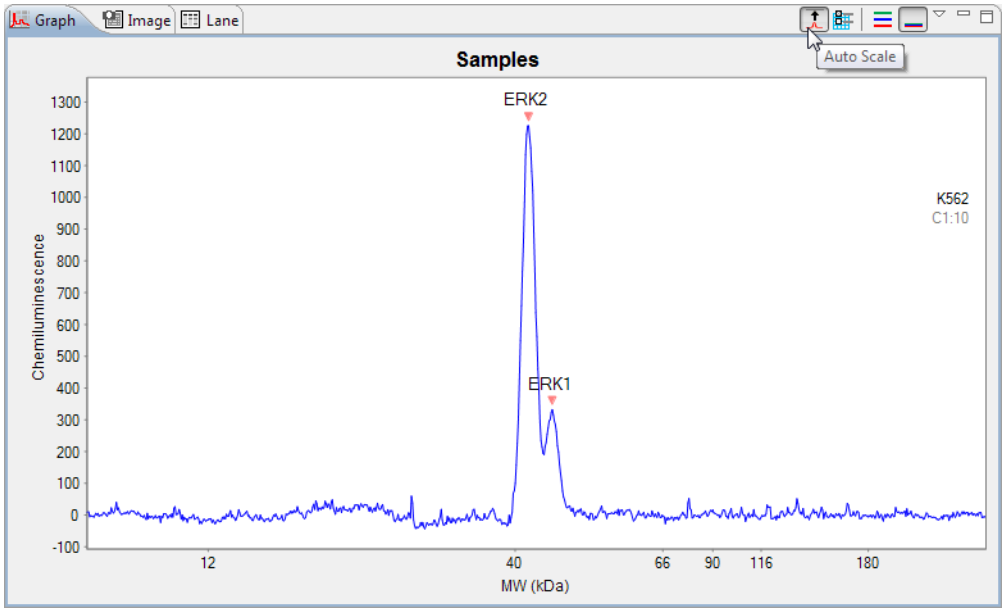
Graph Options

Stack the Plots

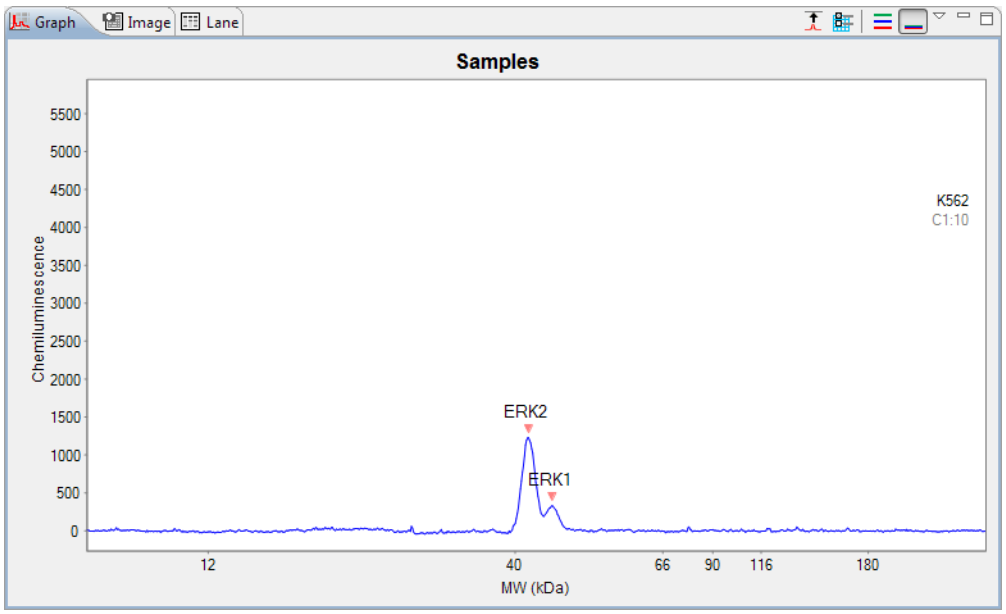
Overlay the Plots

Autoscaling the Electropherogram

Click the **Autoscale** button to scale the y-axis to the largest peak in the electropherogram.



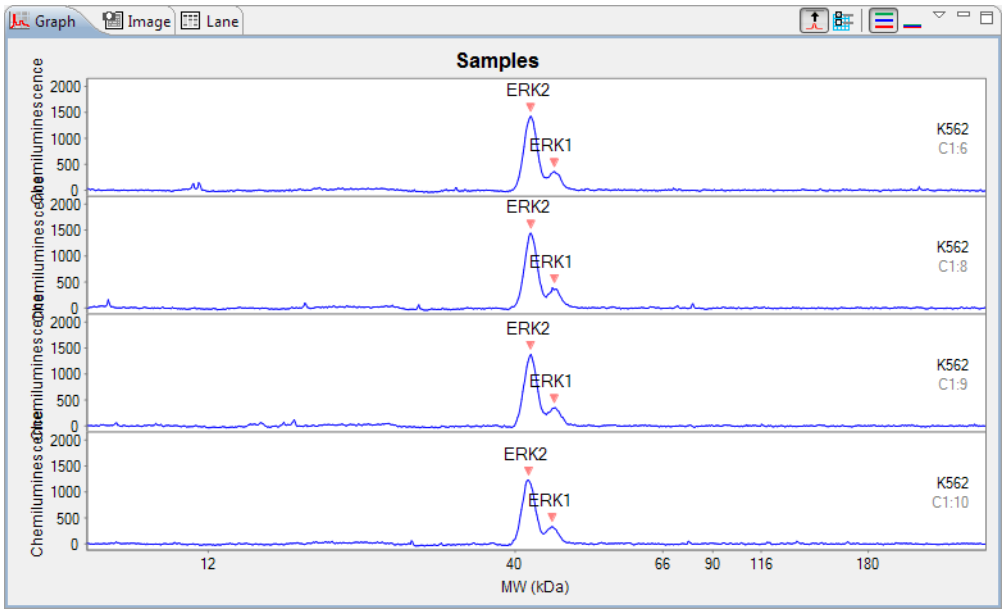
Click the **Autoscale** button again to return to default scaling.



Stacking Multiple Electropherograms

Electropherogram data for multiple capillaries can be stacked vertically in the graph pane for comparison. To do this:

1. Click **Single View**.
2. Select multiple rows in the experiment pane.
3. Click the **Stack the Plots** button. The individual electropherograms for each row selected will stack in the graph pane.

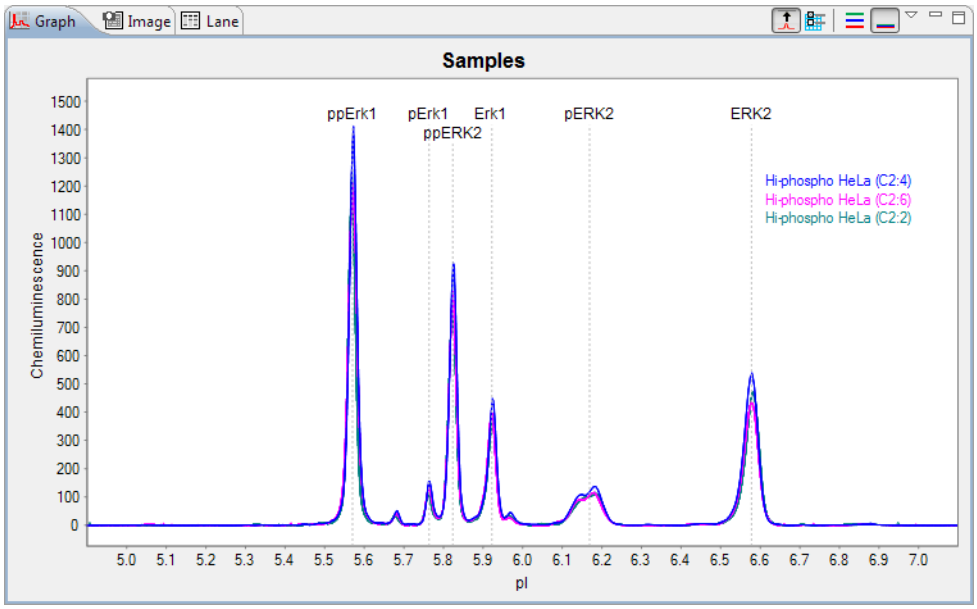


Users can customize the colors used for the stacked plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 392.

Overlaying Multiple Electropherograms

The electropherogram data for multiple capillaries can also be overlaid on top of each other for comparison in the graph pane. To do this:

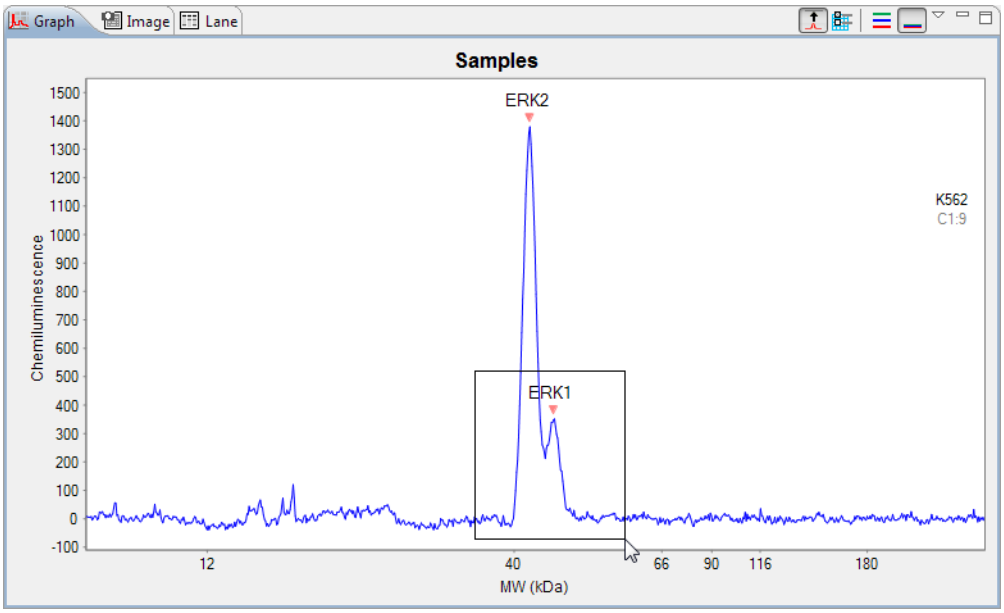
1. Click **Single View**.
2. Select multiple rows in the experiment pane.
3. Click the **Overlay the Plots** button. The individual electropherograms for each row selected will overlay in the graph pane.



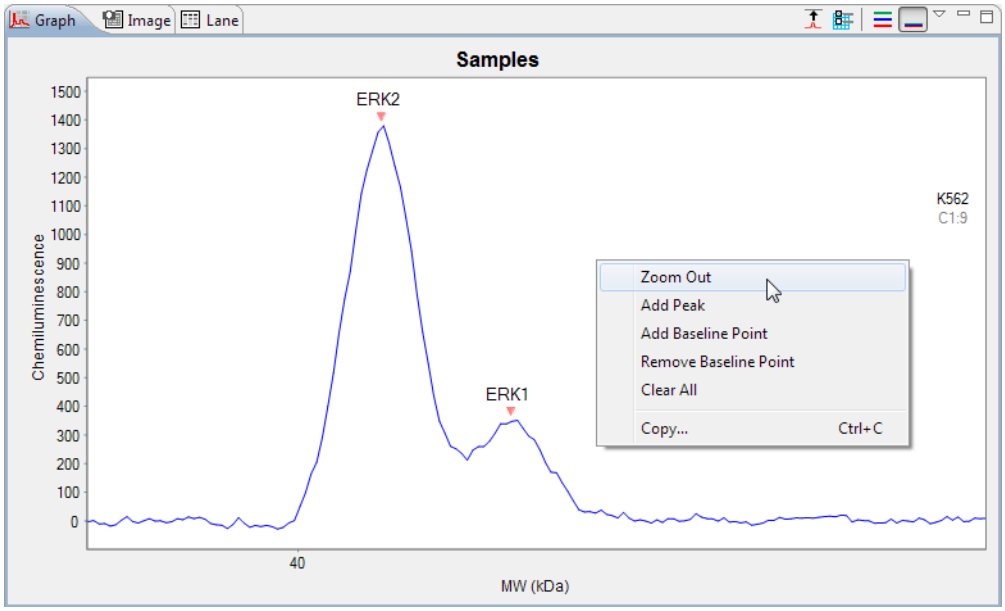
Users can customize the colors used for the overlay plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 392.

Zooming

To zoom in on a specific area of the electropherogram, hold the mouse button down and draw a box around the area with the mouse:

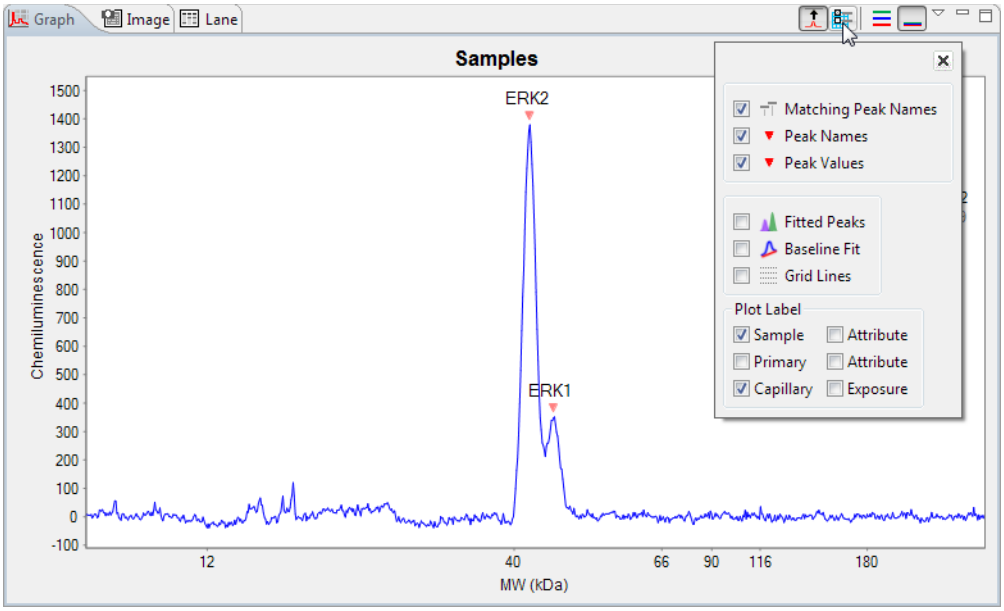


To return to default scaling, right click in the electropherogram and click **Zoom Out**.



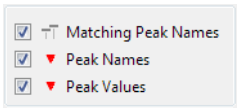
Customizing the Data Display

Electropherogram peak labels, plot labels and display options can be customized by the user. To do this, select the **Graph Options** button.

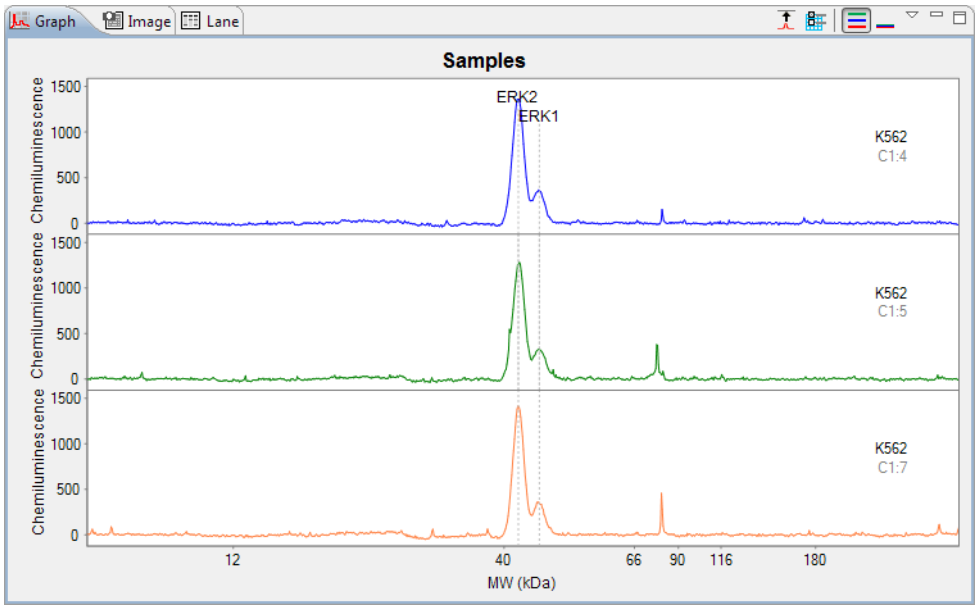


Peak Labels

You can customize the labels used to identify peaks in the electropherogram with these options:

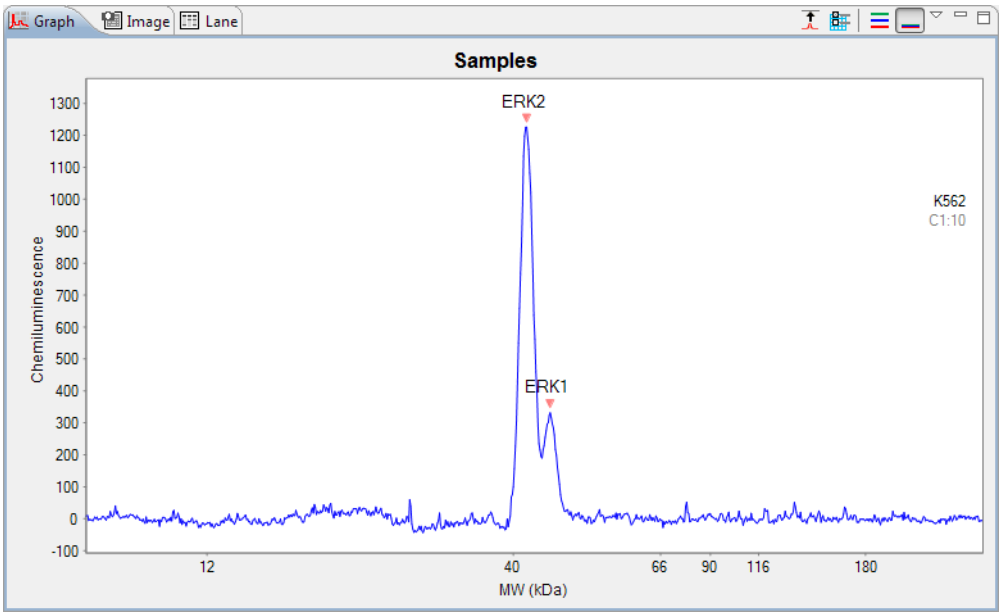


Matching Peak Names - Checking this box will draw vertical lines through each named peak. Using this option with Stack the Plots or Overlay the Plots features is useful for visual comparison of named peaks across multiple capillaries.



- **Peak Names** - Checking this box will display peak name labels on all named peaks in the electropherogram.

NOTE: If more than one peak label option is selected, peak name labels will always be used for named peaks.

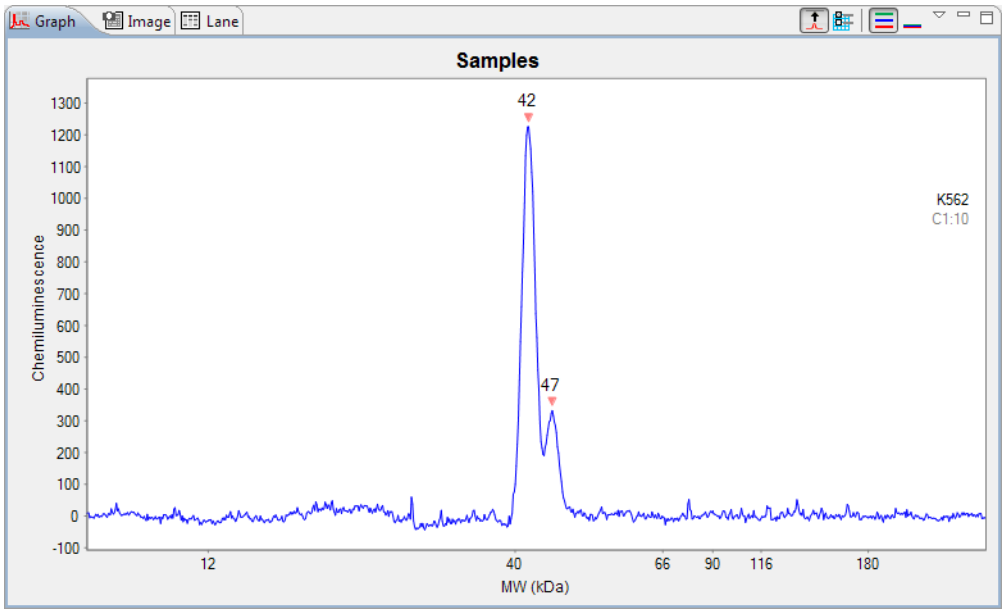


- **Peak Values** - Checking this box will display the molecular weight labels on all peaks in the electropherogram.

NOTES:
When viewing standards and registration data, this option is called Peak Positions. Labels displayed are peak positions rather than molecular weight.

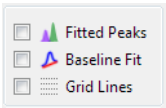
If more than one peak label option is selected, peak name labels will always be used for named peaks.

The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.



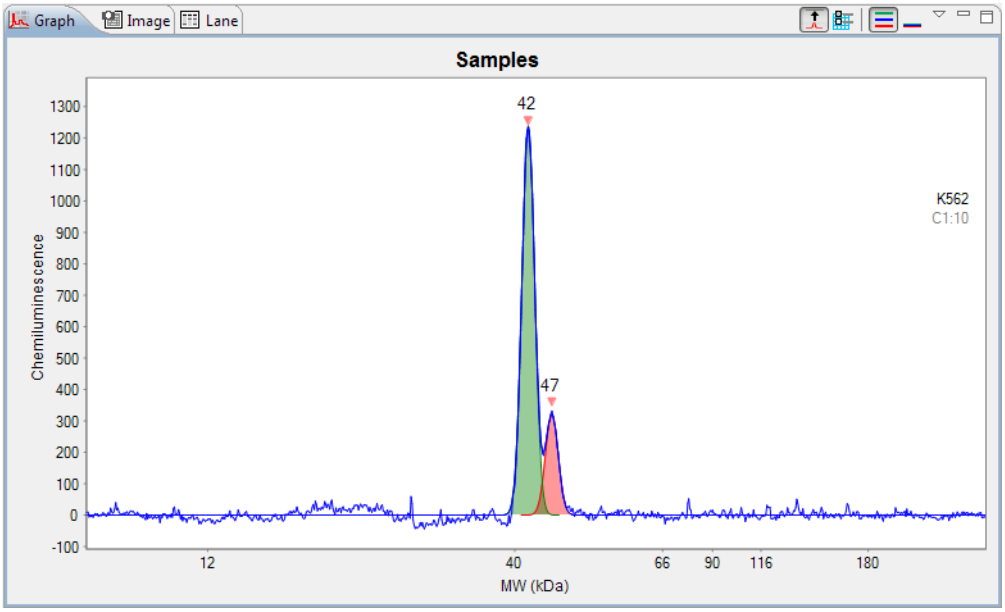
Baseline and Grid Options

You can view the calculated baseline fit, peak integration and show grid lines with these options.



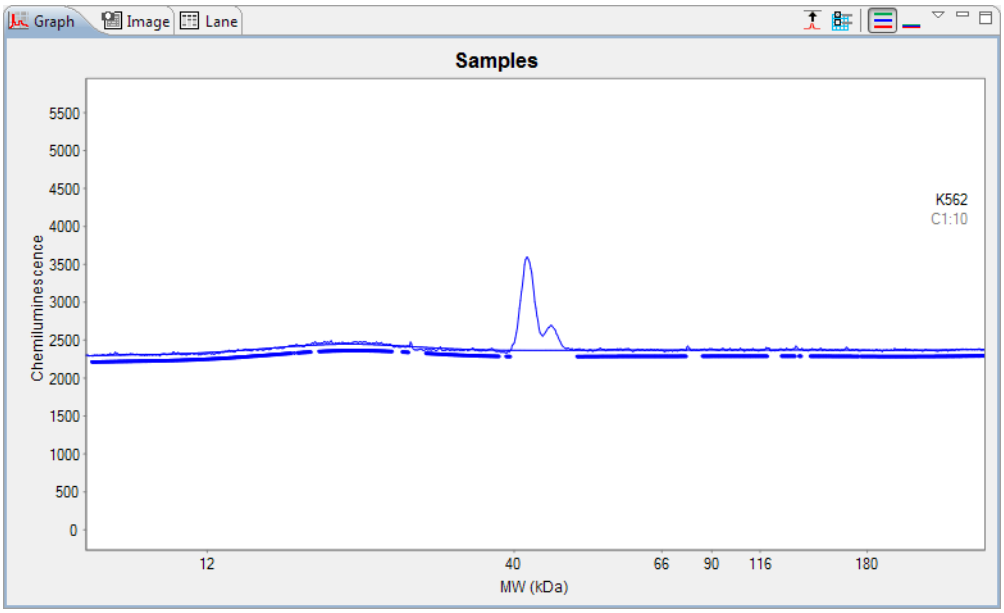
- **Fitted peaks** - Checking this box will display how the peaks were fit by the software.

NOTE: This option is only available for sample data.

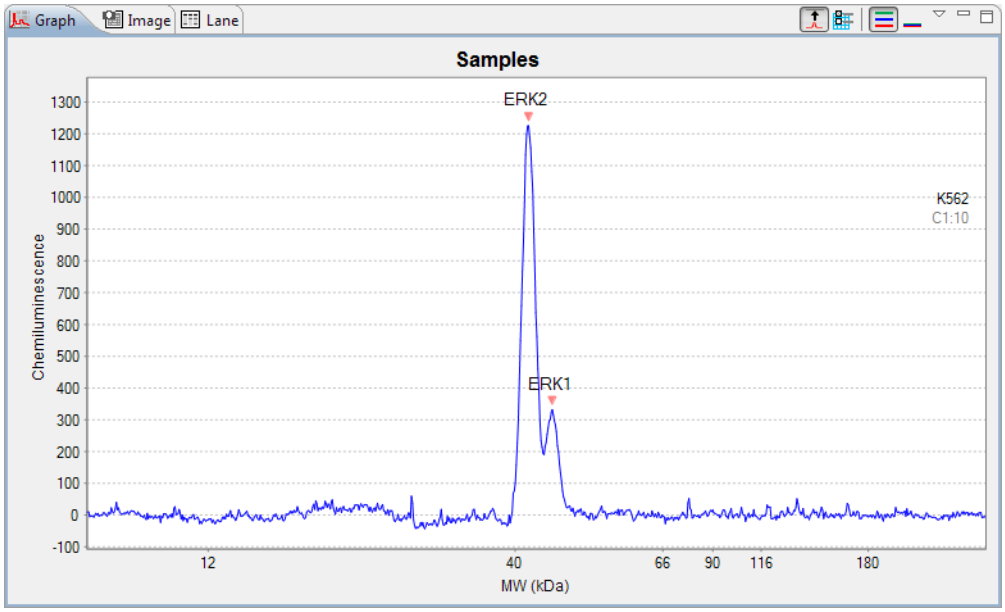


- **Baseline Fit** - Checking this box will display the calculated baseline for the peaks. Baseline points will also display for regions of the electropherogram considered to be at baseline.

NOTE: This option is only available for sample data.

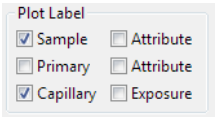


- **Grid Lines** - Checking this box will display grid lines in the graph area.



Plot Labels

You can customize the plot labels displayed on the electropherogram with these options.

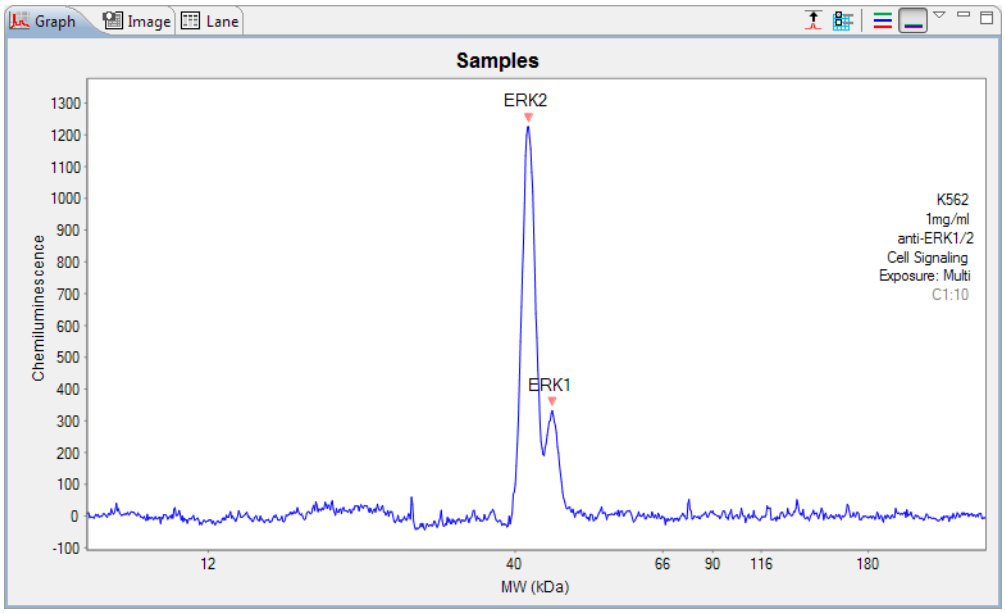


Plot labels are shown on the right side of the graph pane.

- **Sample** - Checking this box will display the sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Checking this box will display the primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** - Checking this box will display the cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
- **Attributes** - Checking this box will display attribute text. If attribute information was entered for samples or primary antibodies in the assay template (Assay screen), they can be selected as plot labels.

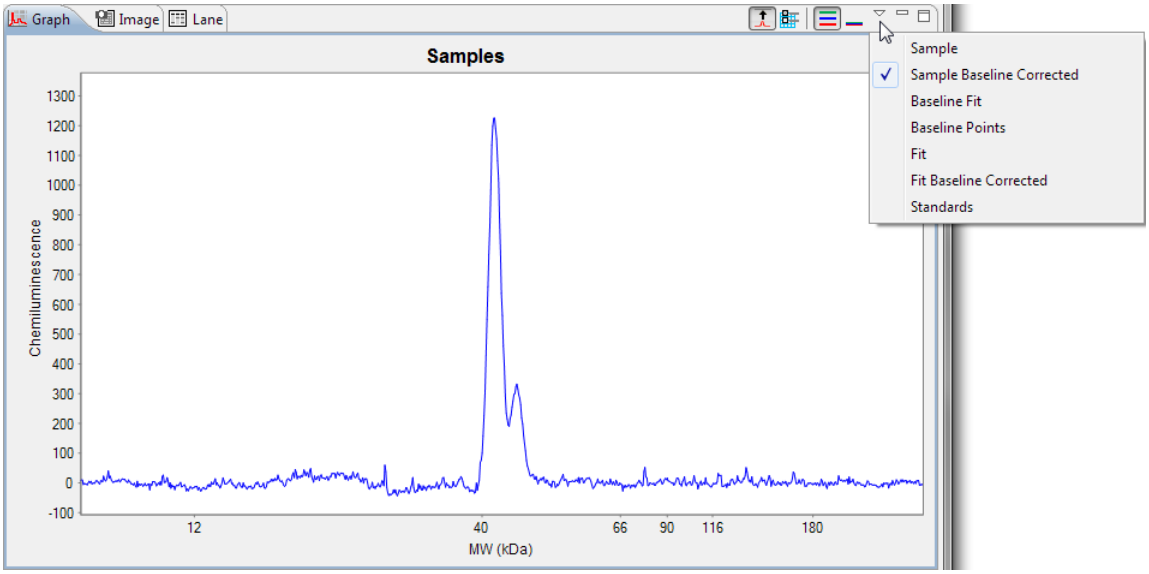
- **Exposure** - Checking this box will display the exposure time(s) used for the data.

The following example shows an electropherogram with all plot labels selected:



Selecting Data Viewing Options

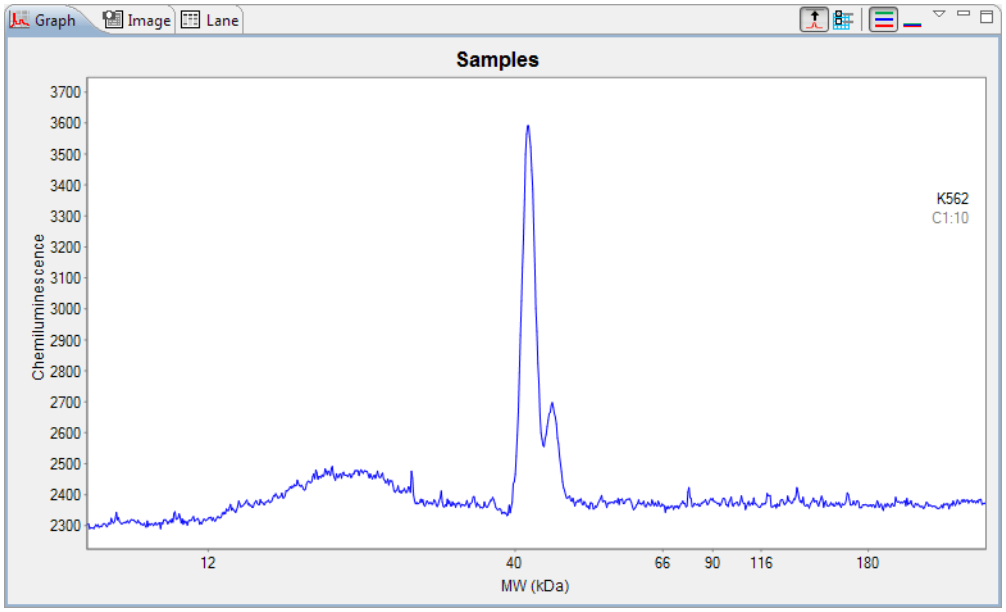
The graph view menu provides multiple user options for changing what type of electropherogram data is displayed. To access the view menu, click the down arrow next to the graph pane toolbar:



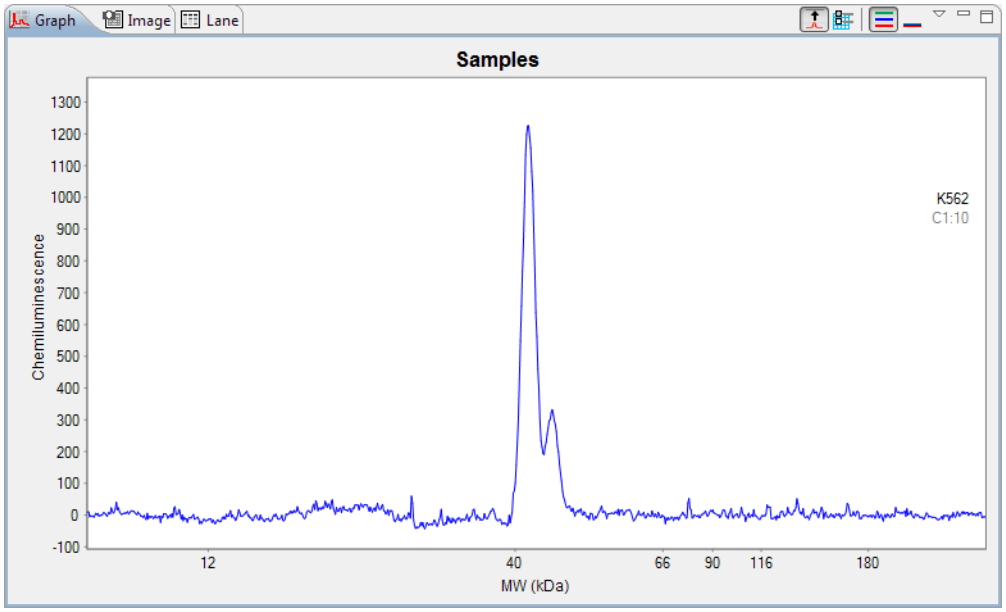
A check mark next to the menu option indicates it is currently selected. Multiple options can be selected at a time.

NOTE: Unless otherwise noted, graph view menu options are available for sample data only.

- **Sample** - Clicking this option will display raw, uncorrected sample data.

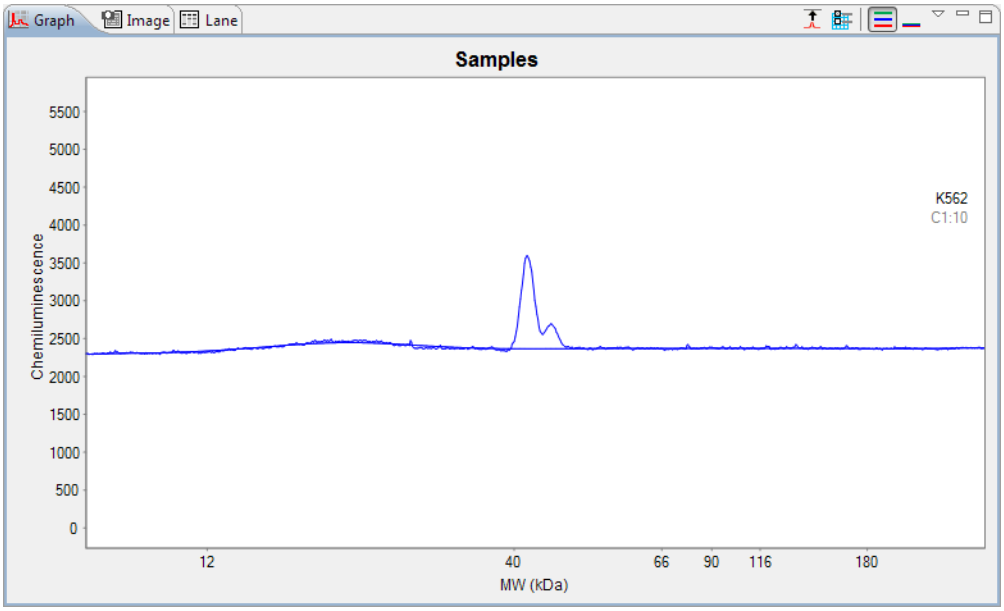


- **Sample Baseline Corrected** - Clicking this option will display sample data with the baseline subtracted (zeroed). This is the default view.



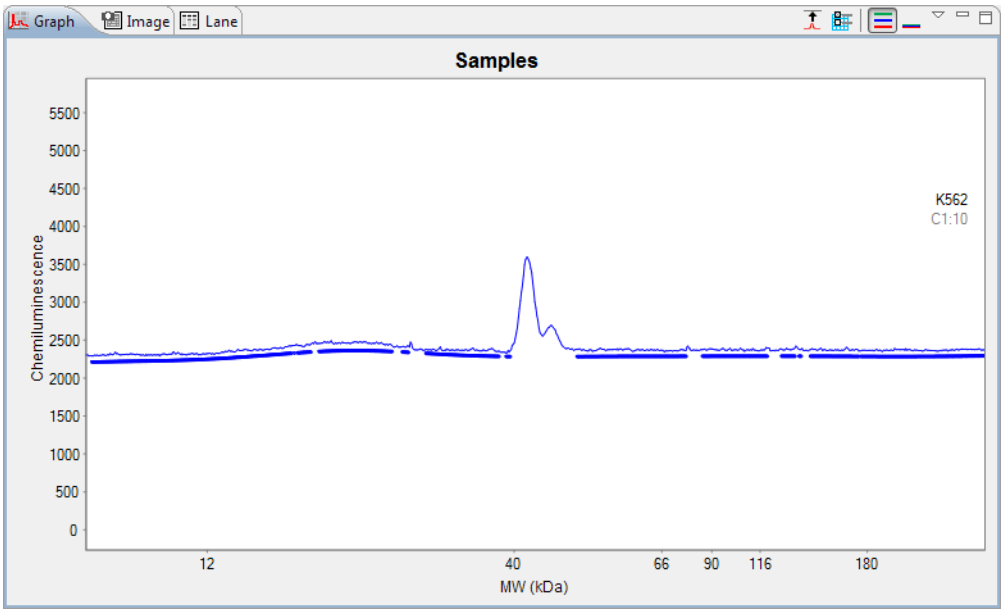
- **Baseline Fit** - Clicking this option will display the calculated baseline for the raw sample data. In the example that follows, both Baseline fit and Sample are selected.

NOTE: This option is selected automatically when Baseline Fit is selected in graph options.

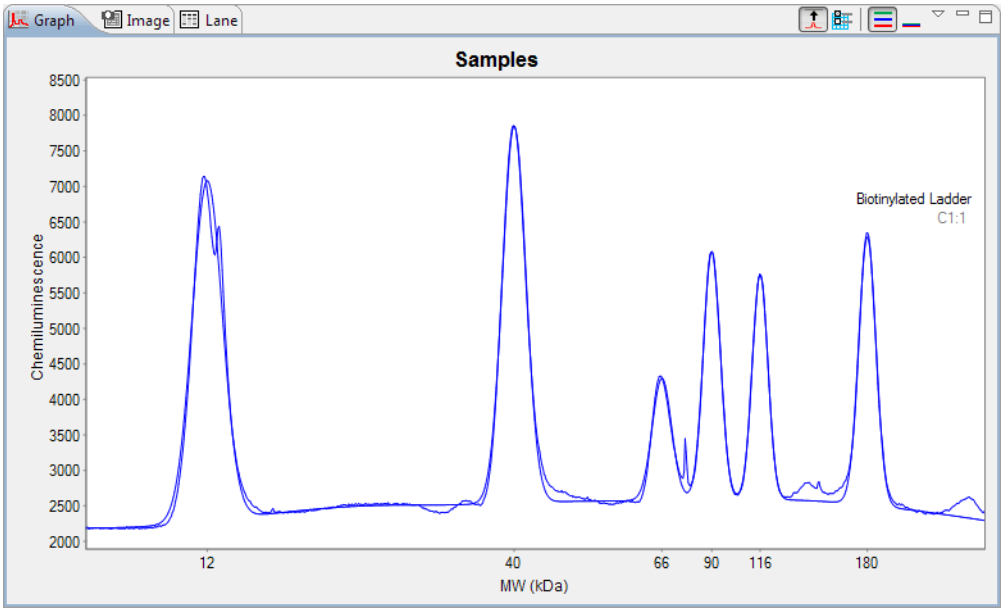


- **Baseline Points** - Clicking this option will display regions of the electropherogram considered to be at baseline. In the example that follows, both Baseline Points and Sample are selected.

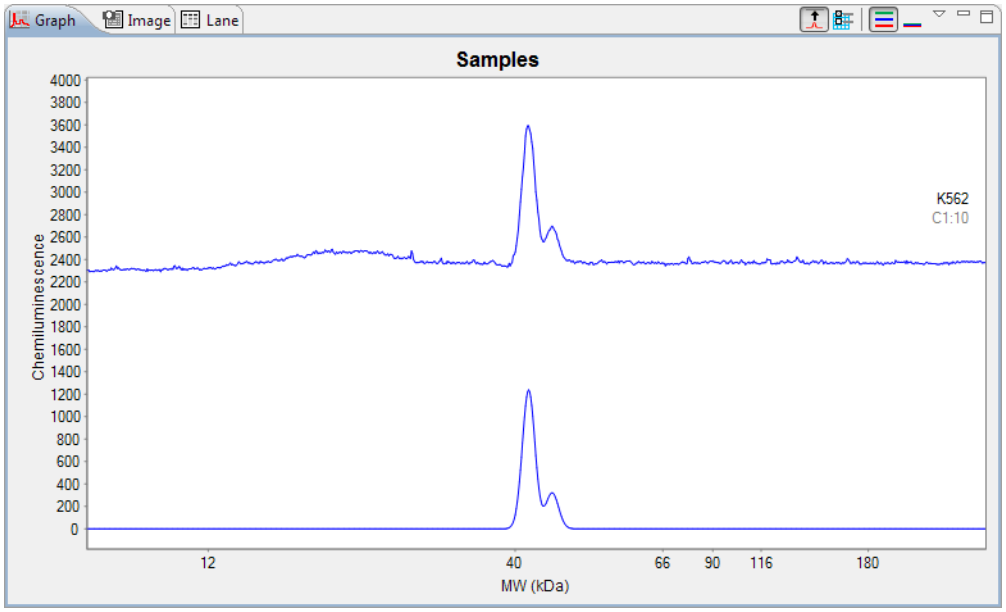
NOTE: This option is selected automatically when Baseline Fit is selected in graph options.



- **Fit** - Clicking this option will display the bounding envelope of the fitted peaks as calculated by the software for the raw sample data. In the example that follows, both Fit and Sample are selected.

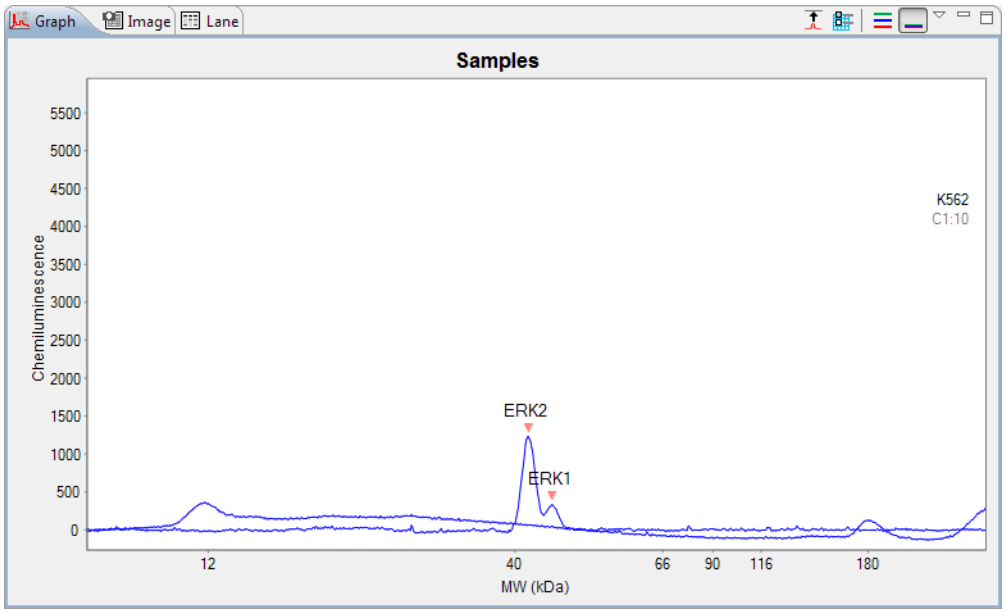


- **Fit Baseline Corrected** - Clicking this option will display the fitted peaks as calculated by the software for the sample baseline corrected data. In the example that follows, both Fit Baseline Corrected and Sample are selected, the fit plot is on the bottom.



NOTE: When viewing standards or registration data, this option is called Standards Fluorescence or Registration Fluorescence, respectively.

- **Standards** - Checking this box aligns the molecular weight of the raw standards data to the sample data and overlays both electropherograms in the graph pane.

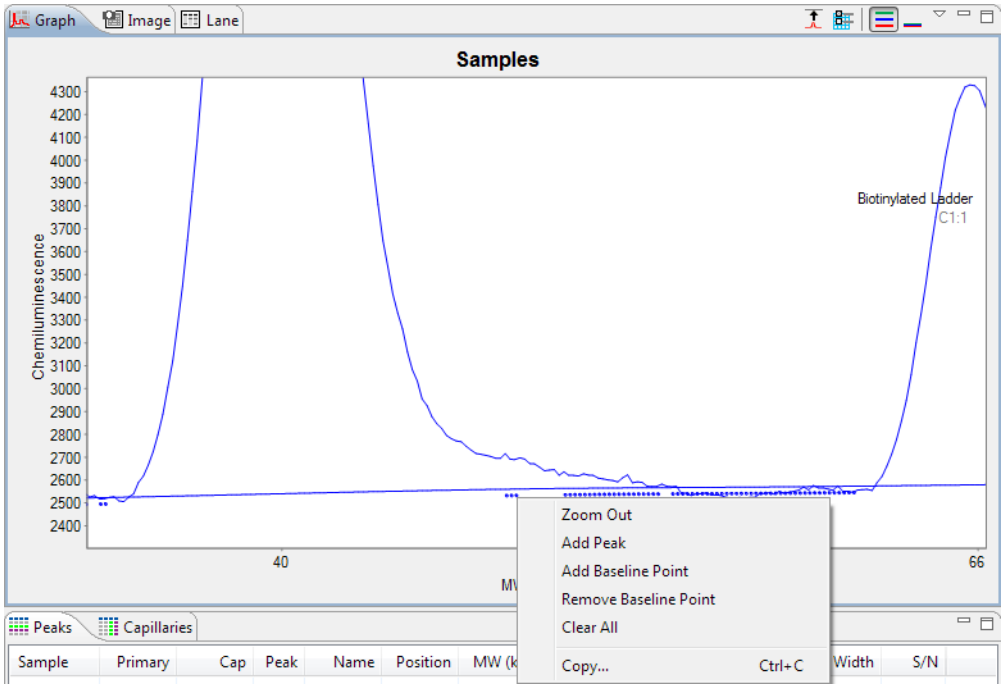


Adding and Removing Baseline Points

Points in the baseline can be added or removed as needed. To do this:

1. Click the **Graph Options** button in the graph pane toolbar and check **Baseline Fit**. This will display baseline points for the raw sample data.
2. Use the mouse to draw a box around the area you want to correct. This will zoom in on the area.

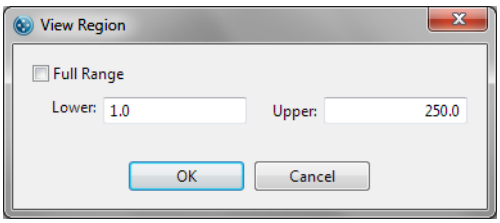
3. Right click a baseline point and click **Add Baseline Point** or **Remove Baseline Point**.



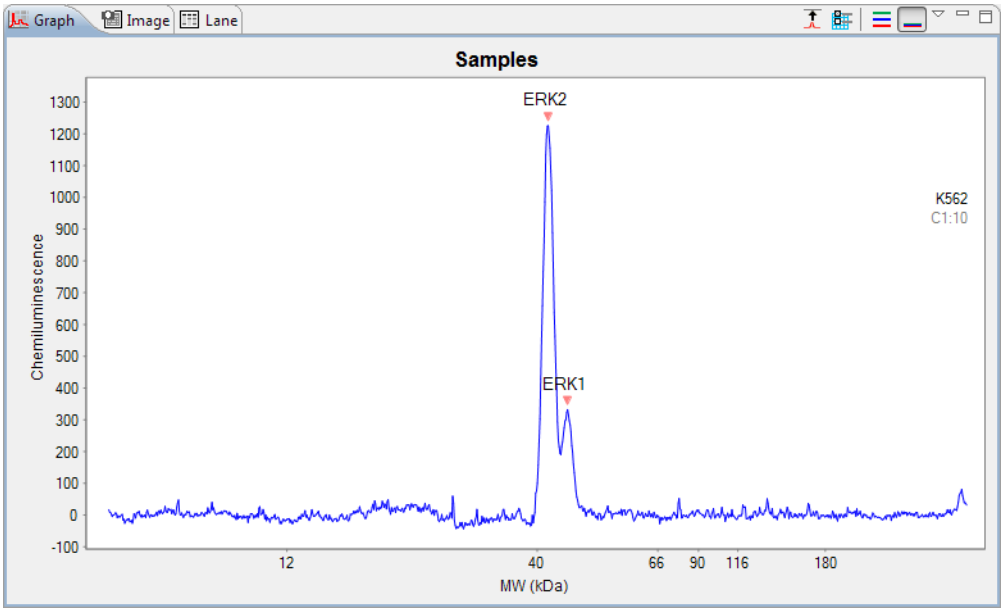
*NOTE: To clear the manual addition or removal of baseline points and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

Selecting the X-Axis Molecular Weight Range

The molecular weight range used for the x-axis can be changed. To do this, Select **View** in the main menu and click **View Region**. The following pop-up window will display:



- **To change the x-axis MW range displayed for the run data** - Enter new values in the Lower or Upper range in molecular weight (kDa) and click **OK**. Electropherogram and virtual blot data will update to display only the data in the entered range.
- **To see the full x-axis MW range included in the run data** - Check **Full Range**. Electropherogram and virtual blot data will update to display the full range of available data.



NOTE: You can change the default x-axis range that Compass uses. For more information, see "Peak Fit Analysis Settings" on page 231.

Closing Run Files

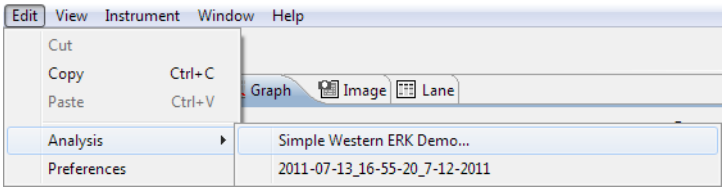
If more than one run file is open, you can close just one file or all the open files at the same time.

- **To close one of multiple open run files** - In the experiment pane, click on one of the sample rows in the file. Next click **File** from the main menu and click **Close**.
- **To close all open run files** - Select **File** from the main menu and click **Close All**.

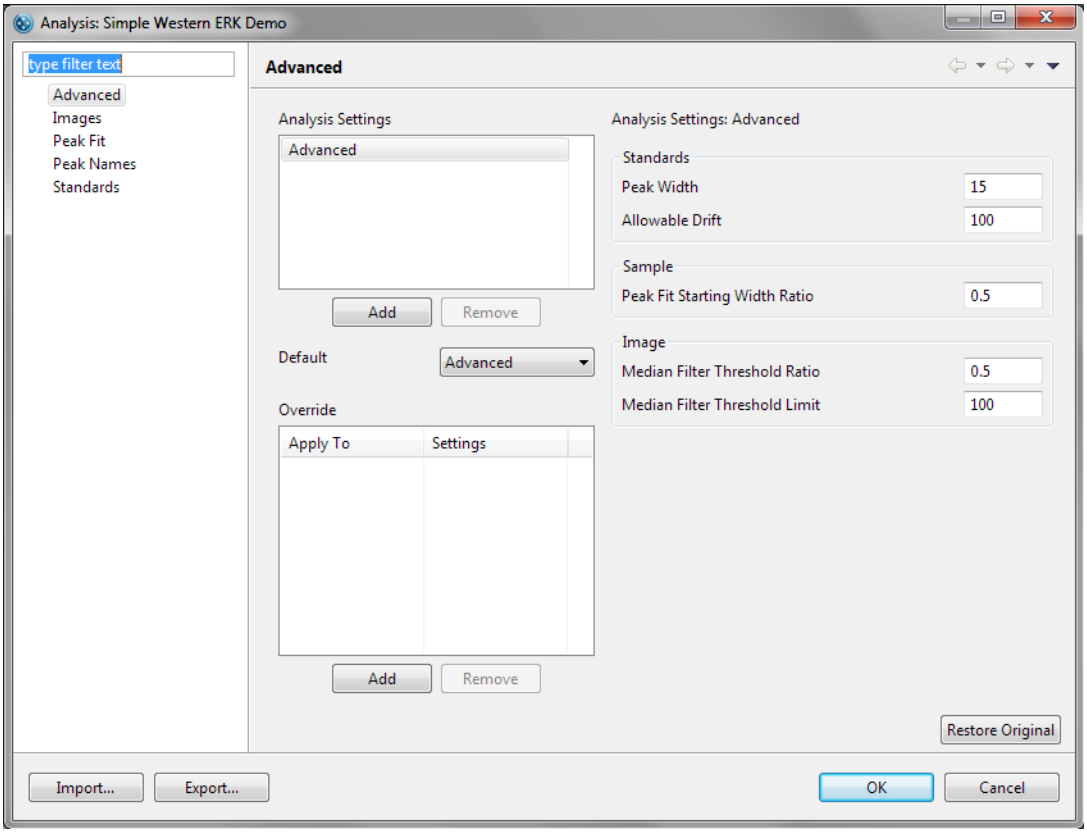
Compass Analysis Settings Overview

Compass has a variety of analysis features and settings that users can modify as needed to enhance run data.

To access these settings, select **Edit** in the main menu and click **Analysis**. If more than one run file is open, select the run file you want to view analysis settings for from the list:



The following screen will display:



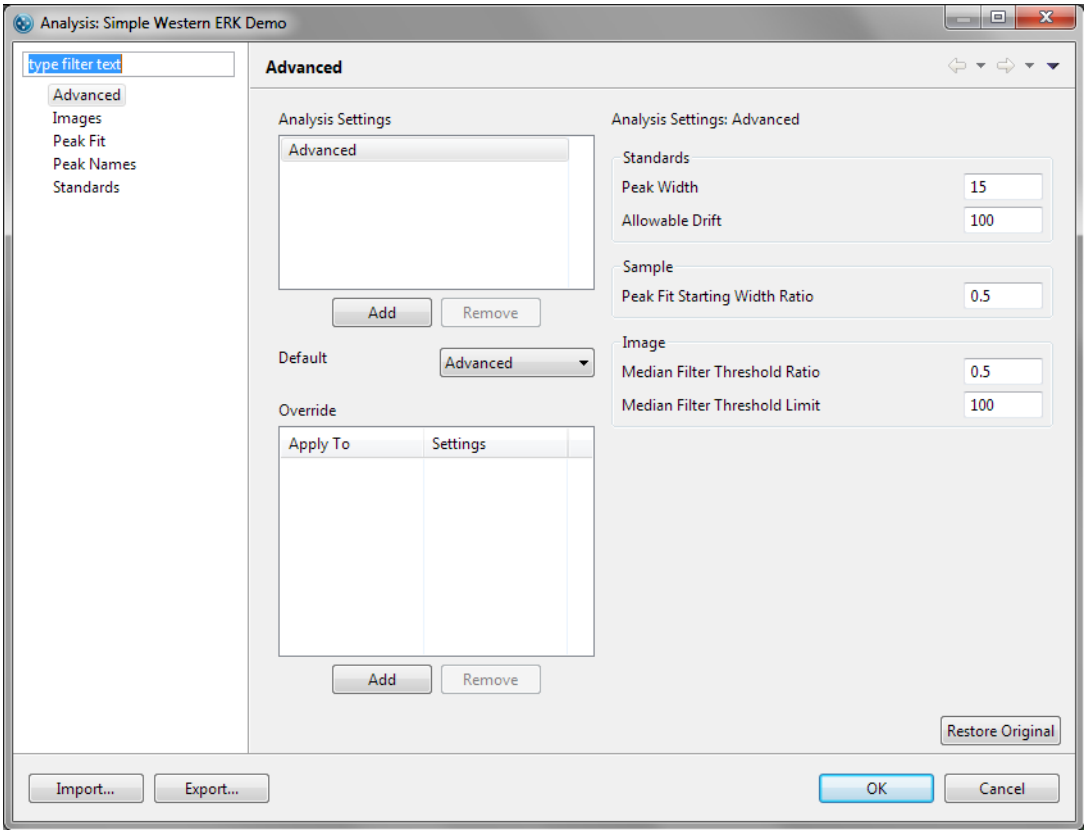
To move between analysis pages in this window, click on an option in the list on the left. The following analysis settings can be user-customized in Compass:

- **Advanced** - Lets you customize analysis settings for samples, standards and image data.
- **Images** - Lets you view the chemiluminescent exposures taken during the run and view data for different exposures in the Analysis screen.
- **Peak Fit** - Lets you customize peak fit settings for sample data.
- **Peak Names** - Lets you enter custom naming settings for sample proteins associated with specific blocking reagents, primary antibodies or attributes and have Compass automatically label the peaks in the run data.
- **Standards** - Lets you customize the molecular weight and positions Compass uses to identify ladder standards, fluorescent standards and registration peaks, as well as change the capillary used for the ladder.

Advanced Analysis Settings

The advanced analysis settings page lets you view and change analysis settings for samples, standards and image data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 261.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 261.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Standards Settings

- **Peak Width** - The approximate width (at full width half max) used to filter out fluorescence artifacts which improves recognition of standards. The default value is 15.
- **Allowable Drift** - The distance in pixels that standards are expected to move compared to their entered positions in the standards analysis page. This setting assists in recognition of standards. The default value is 100.

Sample Settings

- **Peak Fit Starting Width Ratio -** Focuses the peak fit towards the peak center and aids the overall peak fitting. The default value is 0.5.

Image Settings

- **Median Filter Threshold Ratio** - Pixel ratio used to filter out camera artifacts. The default value is 0.5.
- **Median Filter Threshold Limit** - Pixel threshold value used to filter out camera artifacts. The default value is 100.

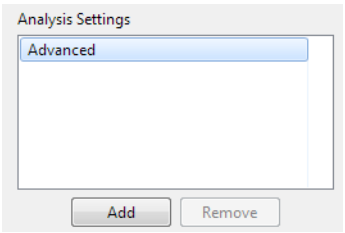
Advanced Analysis Settings Groups

Advanced analysis settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
ProteinSimple recommends using the Compass default values for advanced analysis settings. These settings are included in the default Advanced group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 261.

Analysis groups are displayed in the analysis settings box:

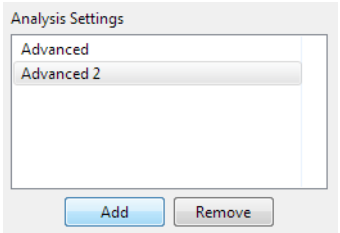


The Advanced group shown contains the Compass default analysis settings. Users can make changes to this group and create new groups.

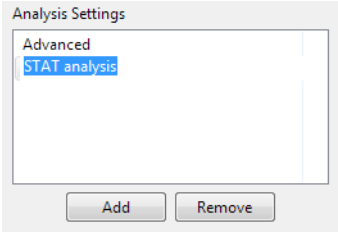
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Analysis Group

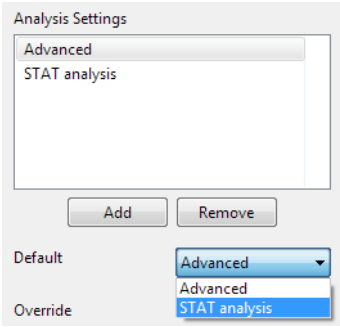
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



3. Click on the new group and enter a new name.



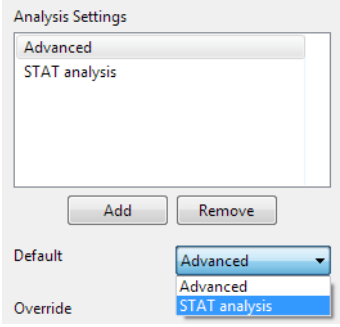
4. Modify standards, sample or image parameters as needed.
5. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.



6. Click **OK** to save changes.

Changing the Default Analysis Group

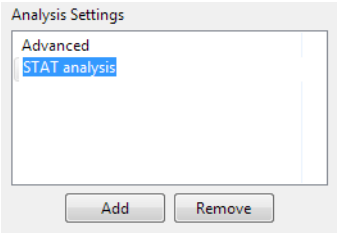
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying an Analysis Group

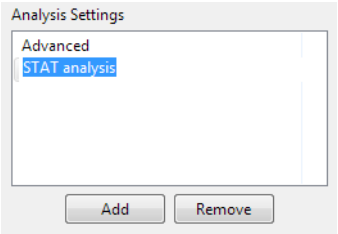
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify standards, sample or image parameters as needed.
4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

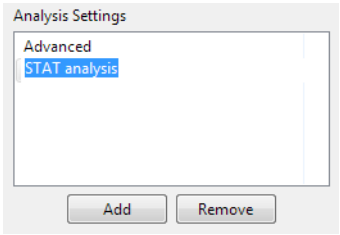
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



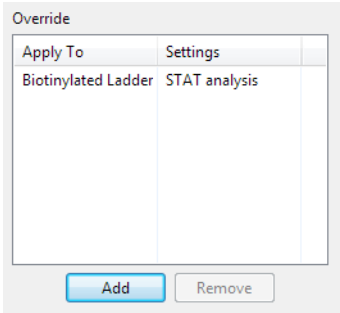
3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

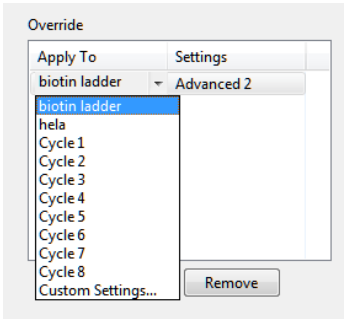
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.



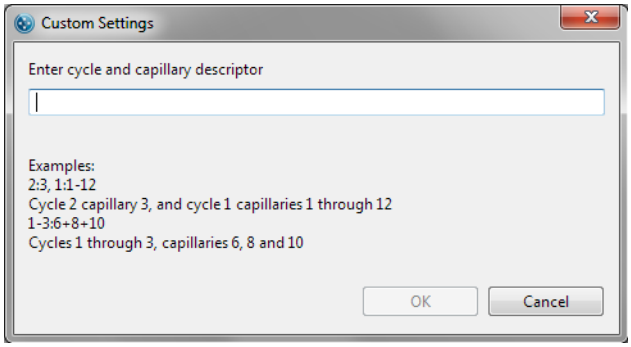
4. Click the cell in the **Apply To** column, then click the down arrow.



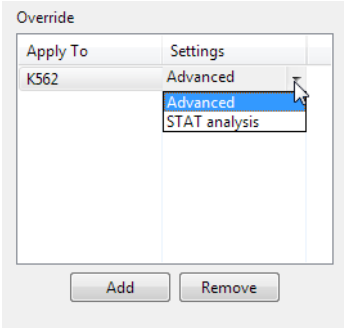
5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.

- **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



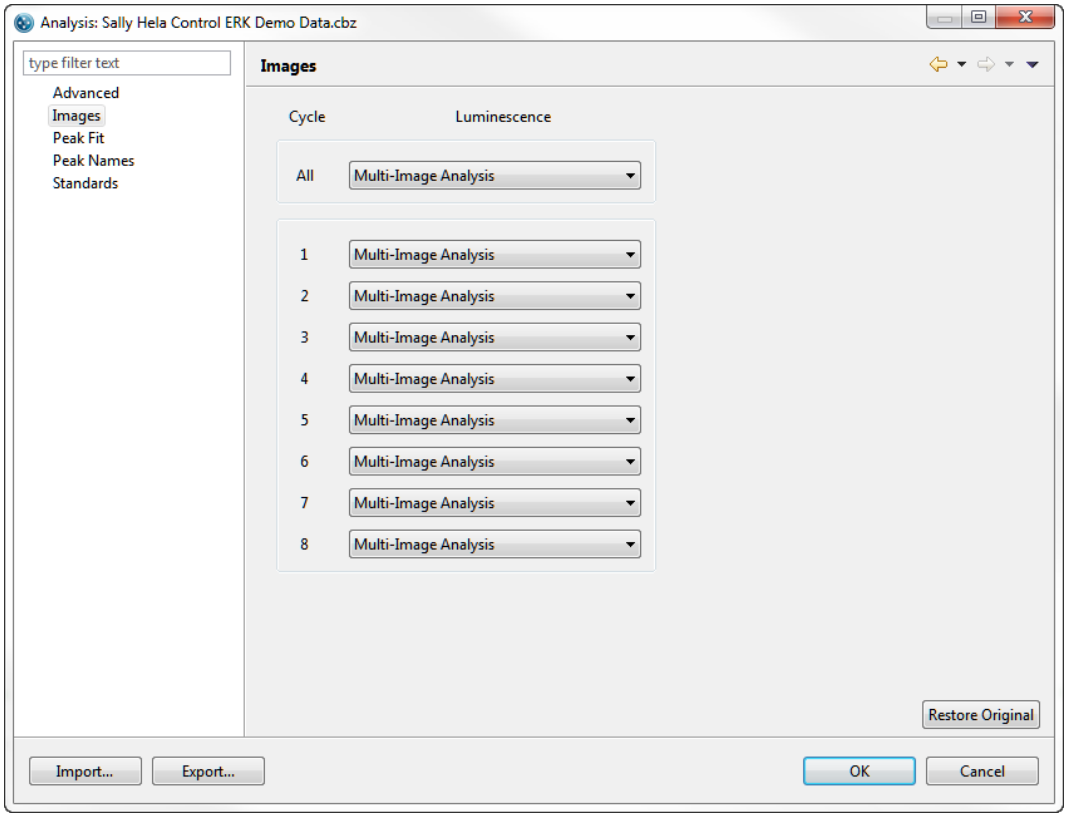
6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Images Analysis Settings

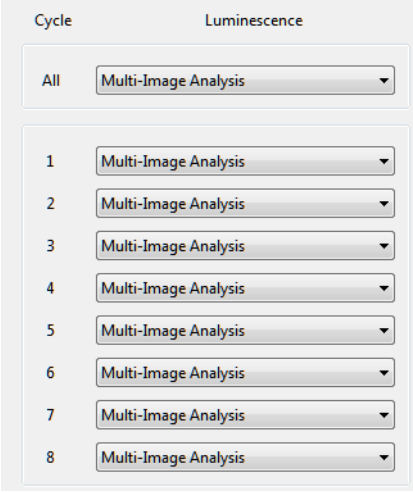
The Images analysis settings page lets you see what chemiluminescent exposures were taken during the run, and view data for different exposures in the Analysis screen. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 261.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 261.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Exposure Settings

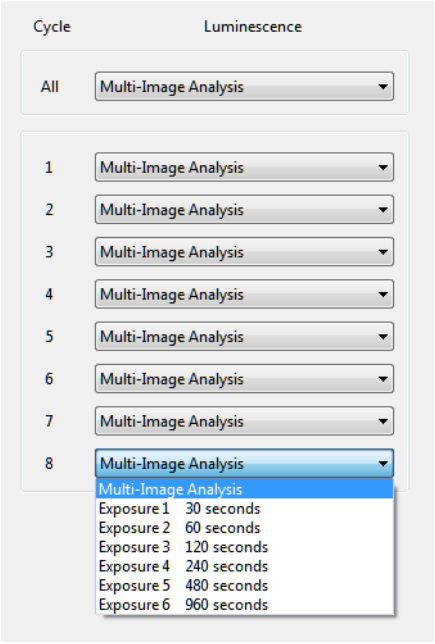
The exposure used for the sample data displayed in the Analysis screen is shown in the All box:



NOTE: Peggy runs up to eight cycles (12 capillaries per cycle). The individual exposure setting selected for a specific cycle is applied for all 12 capillaries in that cycle unless selected for All cycles.

- **Multi-Image Analysis** - Sample data displayed in the Analysis screen is compiled from all exposures taken during the run and utilized to calculate the chemiluminescent signal output at time zero of the chemiluminescent reaction. This calculation represents the kinetics of the chemiluminescent reaction and helps to eliminate signal burn out that may occur with stronger signals and longer exposure times.
- **Exposure #** - Sample data displayed in the Analysis screen is for this specific exposure only.

To see the number of exposures and exposure times used for the run data, click the arrow in the drop down list next to **All**.

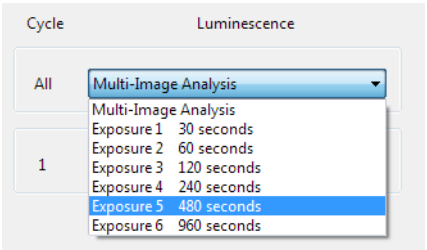


NOTE: The number of exposures taken and exposure times shown were specified in the assay protocol (Assay screen) prior to executing the run and cannot be modified.

Changing the Sample Data Exposure Displayed

The exposure used for the sample data displayed in the Analysis screen can be changed. To do this:

1. Select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.
2. Click the arrow in the drop down list next to **All** and select an exposure setting:

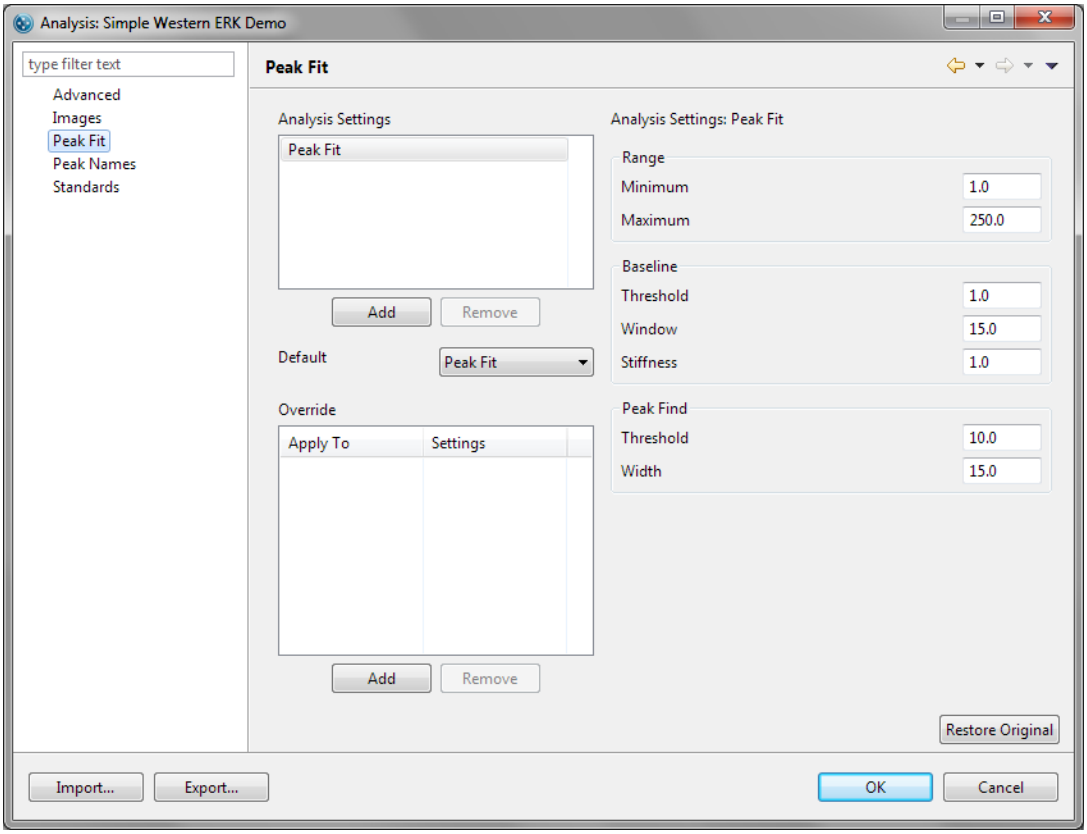


3. Click **OK** to save changes and exit. Sample data for the exposure selected will display in the Analysis screen.

Peak Fit Analysis Settings

The peak fit analysis settings page lets you view and change peak fit settings for sample data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 261.

- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 261.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Range Settings

- **Minimum** - The molecular weight value (in kDa) below which peaks will not be identified. This value will also be used as the default lower MW range for the data displayed in the electropherogram and virtual blot. The default value is 1 kDa.
- **Maximum**: The molecular weight value (in kDa) above which peaks will not be identified. This value will also be used as the default upper MW range for the data displayed in the electropherogram and virtual blot. The default value is 250 kDa.

Baseline Settings

- **Threshold** - The variance, or roughness, in a baseline data segment below which a point will be called part of the baseline. The default value is 1.0.
- **Window** - How long baseline data segments are expected to be in pixels. Shorter segments will allow the baseline to follow plateau sections of the signal. The default value is 15.
- **Stiffness** - The amount the baseline is allowed to vary from a straight line. Settings between 0.1 and 1.0 make the baseline fit closer to a straight line. Settings from 1.0 to 10.0 will make the baseline fit follow the data more closely. The default value is 1.0.

Peak Find Settings

- **Threshold** - The minimum signal to noise ratio required for a peak to be identified. A setting of 1.0 will detect many peaks, a setting of 10.0 will detect fewer peaks. The default value is 10.0.
- **Width** - The approximate peak width (at full width half max) in pixels used to detect peaks. The minimum value for this setting is 3.0. Larger widths help to eliminate the detection of shoulder peaks and noise peaks. The default value is 15.0.

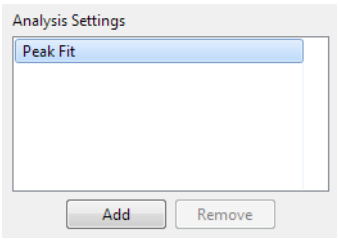
Peak Fit Analysis Settings Groups

Peak fit settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
ProteinSimple recommends using the Compass default values for peak fit analysis settings. These settings are included in the default Peak Fit group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 261.

Peak fit groups are displayed in the analysis settings box:

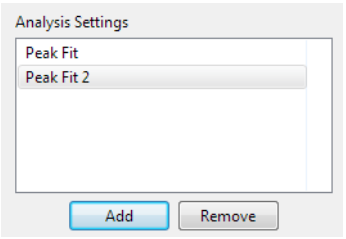


The Peak Fit group shown contains the Compass default analysis settings. Users can make changes to this group and create new groups.

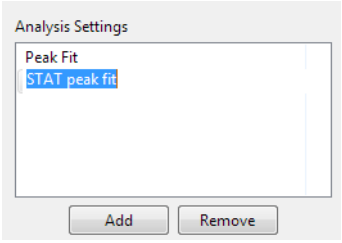
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Peak Fit Group

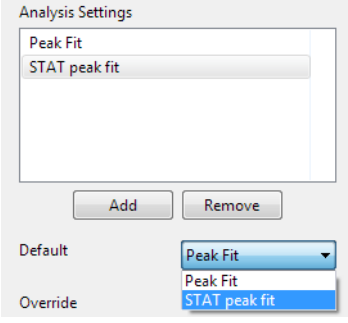
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



3. Click on the new group and enter a new name.



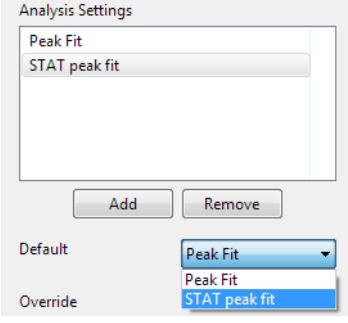
4. Modify range, baseline or peak find parameters as needed.
5. To use the new group as the default peak fit settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Peak fit settings in the new group will then be applied to the run data.



6. Click **OK** to save changes.

Changing the Default Peak Fit Group

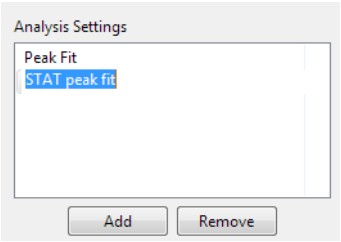
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Peak fit settings in the group selected will be applied to the run data.

Modifying a Peak Fit Group

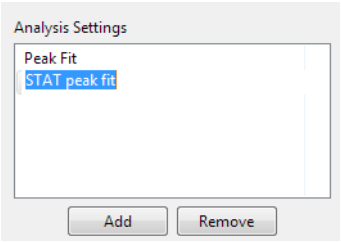
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify range, baseline or peak find parameters as needed.
4. Click **OK** to save changes. The new peak fit settings will be applied to the run data.

Deleting a Peak Fit Group

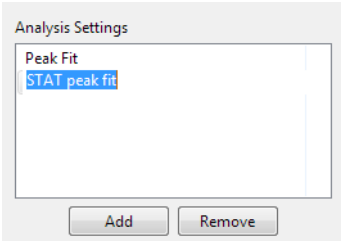
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



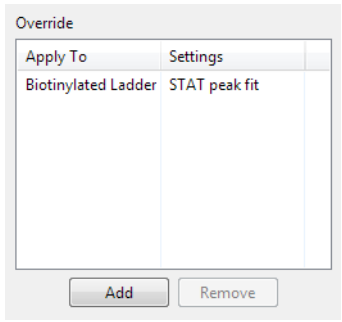
3. Click **OK** to save changes.

Applying Peak Fit Groups to Specific Run Data

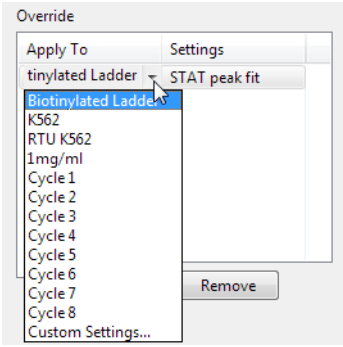
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of peak fit groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.



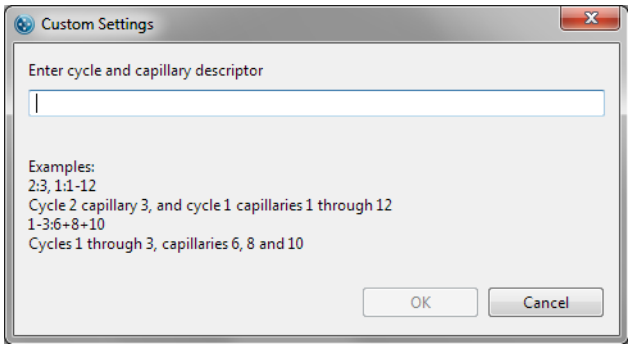
4. Click the cell in the **Apply To** column, then click the down arrow.



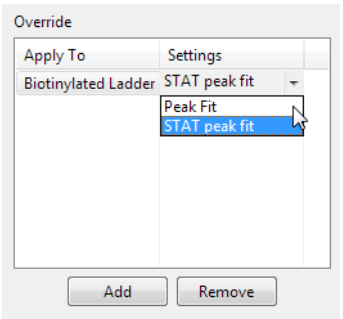
5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.

- **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
- **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the peak fit group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

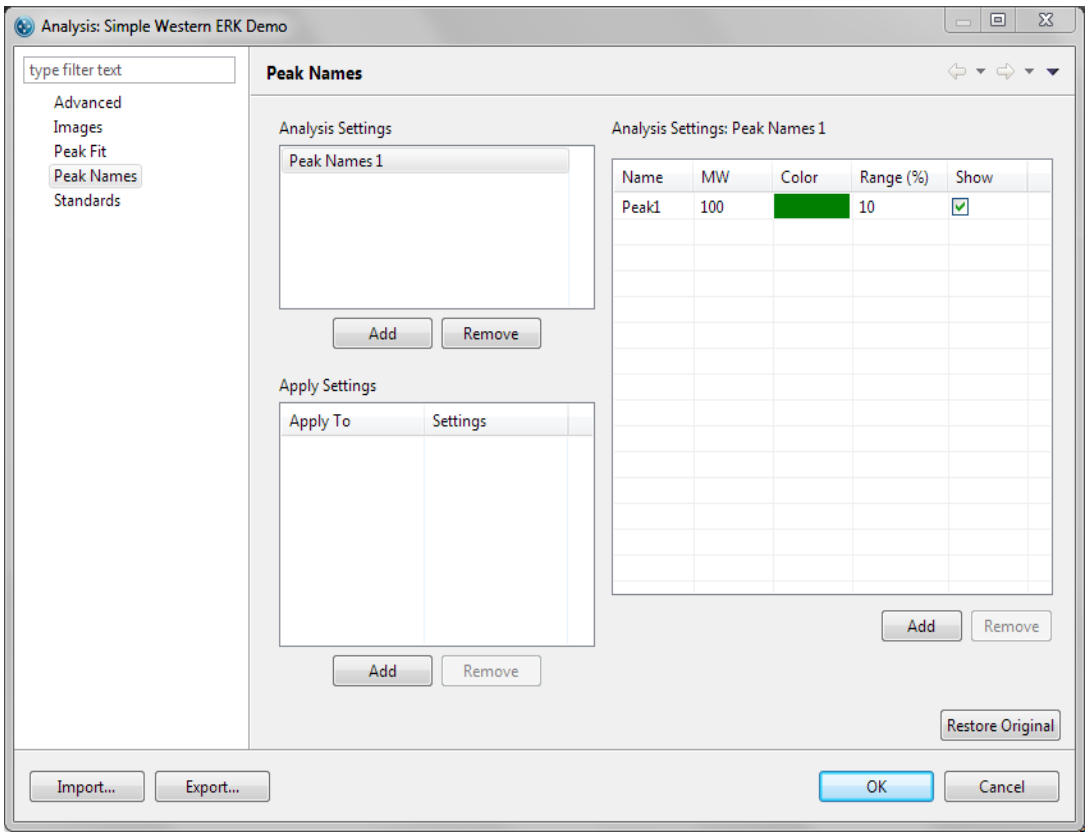


7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Peak Names Settings

The peak names analysis settings page lets you enter custom naming settings for sample proteins. Compass can automatically label sample peaks in the run data associated with specific blocking reagent names, primary antibody names or attributes. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 261.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 261.
- Click **Restore Original** to restore Compass default settings.

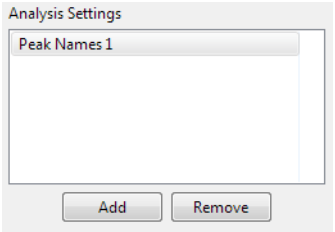
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Peak Names Analysis Settings Groups

Peak name settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, blocking reagent names or primary antibody names and attributes in the run data.

NOTE: Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 261.

Peak name groups are displayed in the analysis settings box:

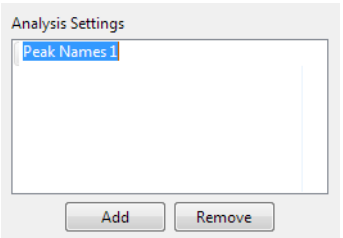


The Peak Names group shown is a Compass template group. Users can make changes to this group and create new groups.

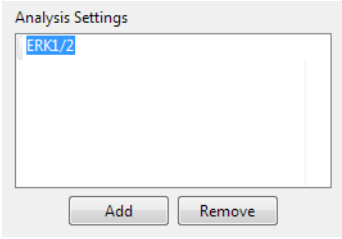
To view settings for a group, click on the group name in the analysis settings box.

Creating a Peak Names Group

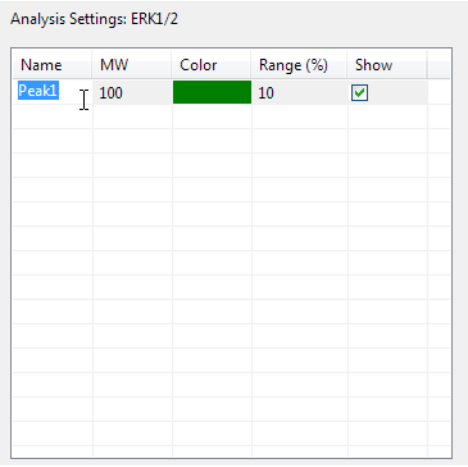
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the **Peak Names 1** template group in the analysis settings box.



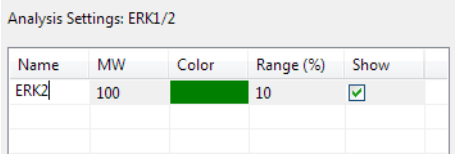
3. Enter a new name for the group.



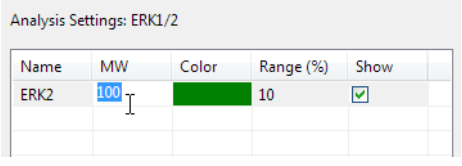
4. Click in the first cell in the **Name** column in the analysis settings peak table.



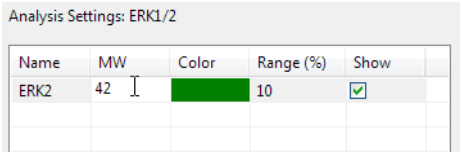
5. Enter a sample protein name associated with the primary antibody used in the run.



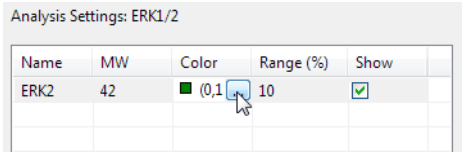
6. Click in the first cell in the **MW** column.



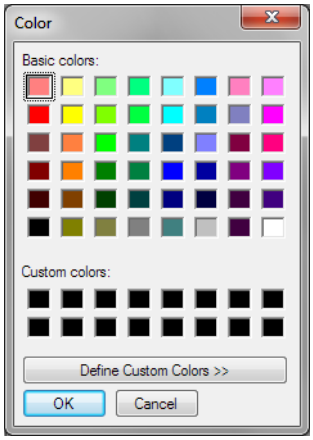
7. Enter the molecular weight (in kDa) for the sample protein.



8. Click in the first cell in the **Color** column, then click the button.

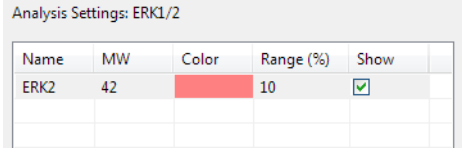


The color selection box will display:

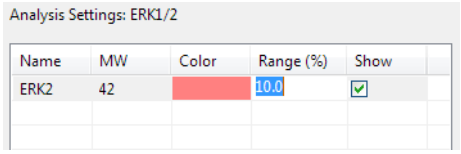


The color selected will be used to identify the sample protein peak in the peaks and capillaries table in the Analysis screen.

9. Click a color or define a custom color and click **OK**. The color selection will update in the table:



10. Click in the first cell in the **Range (%)** column.



11. Enter a range window for the MW entered. Compass will automatically name peaks found within this percent of the molecular weight. For example, if the molecular weight entered is 40 kDa and a 10% range is used, all peaks between 36 and 44 kDa will be identified with this peak name.

NOTE: The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

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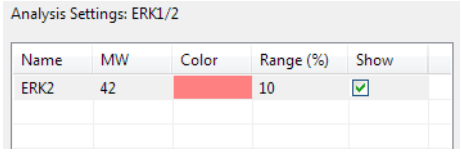
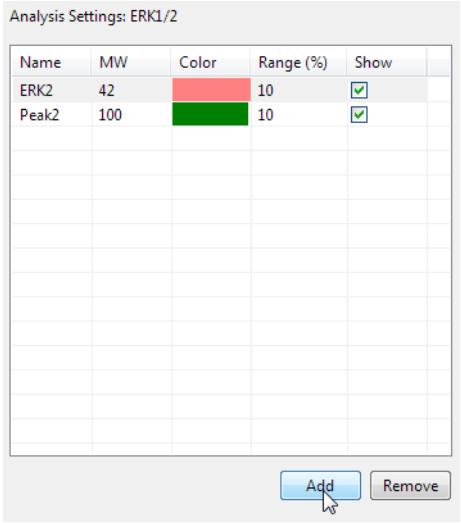
12. Select the checkbox in the first cell of the **Show** column. This will turn peak naming on for the sample protein.

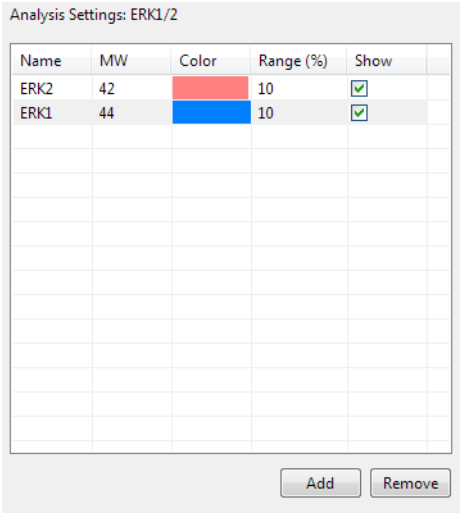


To turn peak naming off for a particular sample protein, deselect the checkbox in the Show column.

13. To add another sample protein, click **Add** under the analysis settings peak table:



14. Repeat the previous steps to enter information for other sample proteins. In the following example, two sample proteins were entered:

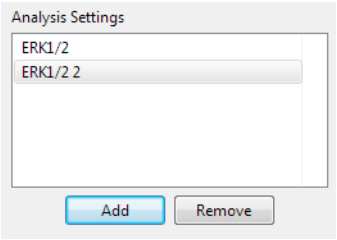


To remove a sample protein, select its row and click **Remove**.

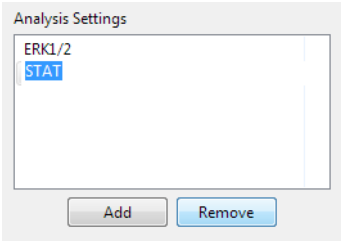
15. Click **OK** to save changes.

Adding Peak Names Groups

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



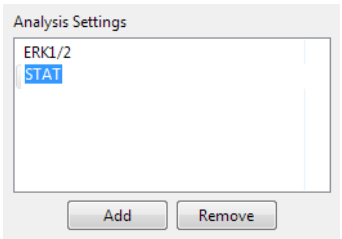
3. Click on the new group and enter a new name.



4. Enter information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 239.
5. Click **OK** to save changes.

Modifying a Peak Names Group

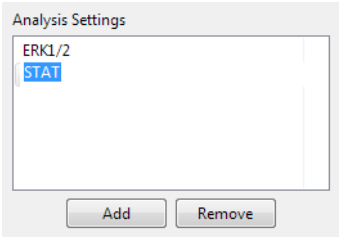
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Change the information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 239.
4. Click **OK** to save changes.

Deleting a Peak Names Group

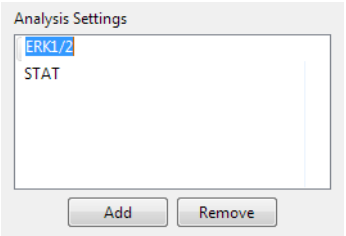
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



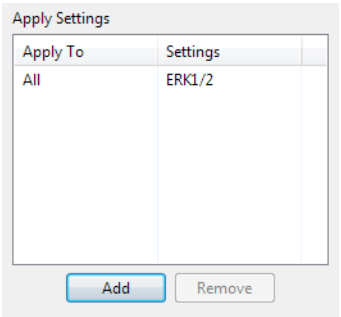
3. Click **OK** to save changes.

Applying Peak Names Groups to Run Data

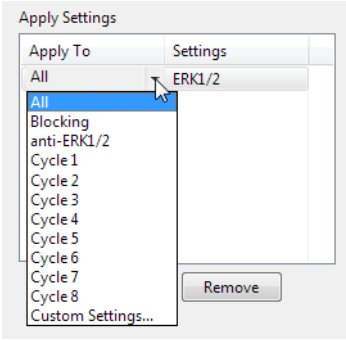
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.

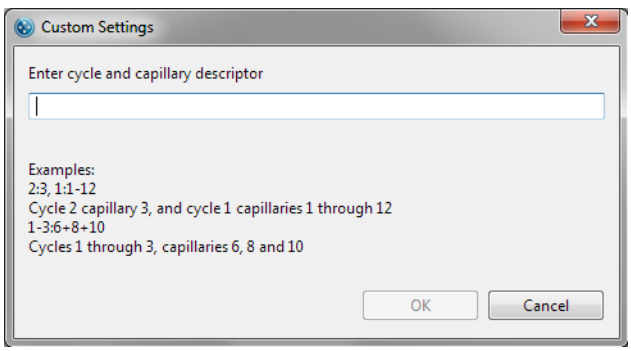


4. Click the cell in the **Apply To** column, then click the down arrow.

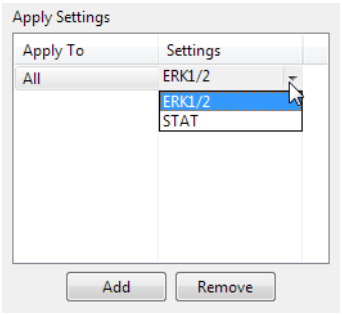


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **All** - When this option is selected, peak names group settings will be applied to all capillaries in the run file.
   - **Blocking reagent name** - All blocking reagent names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Blocking will be shown. Select a name to apply group settings to all capillaries that use this reagent name in the run file.
   - **Primary antibody names** - All primary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
   - **Attributes** - All primary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

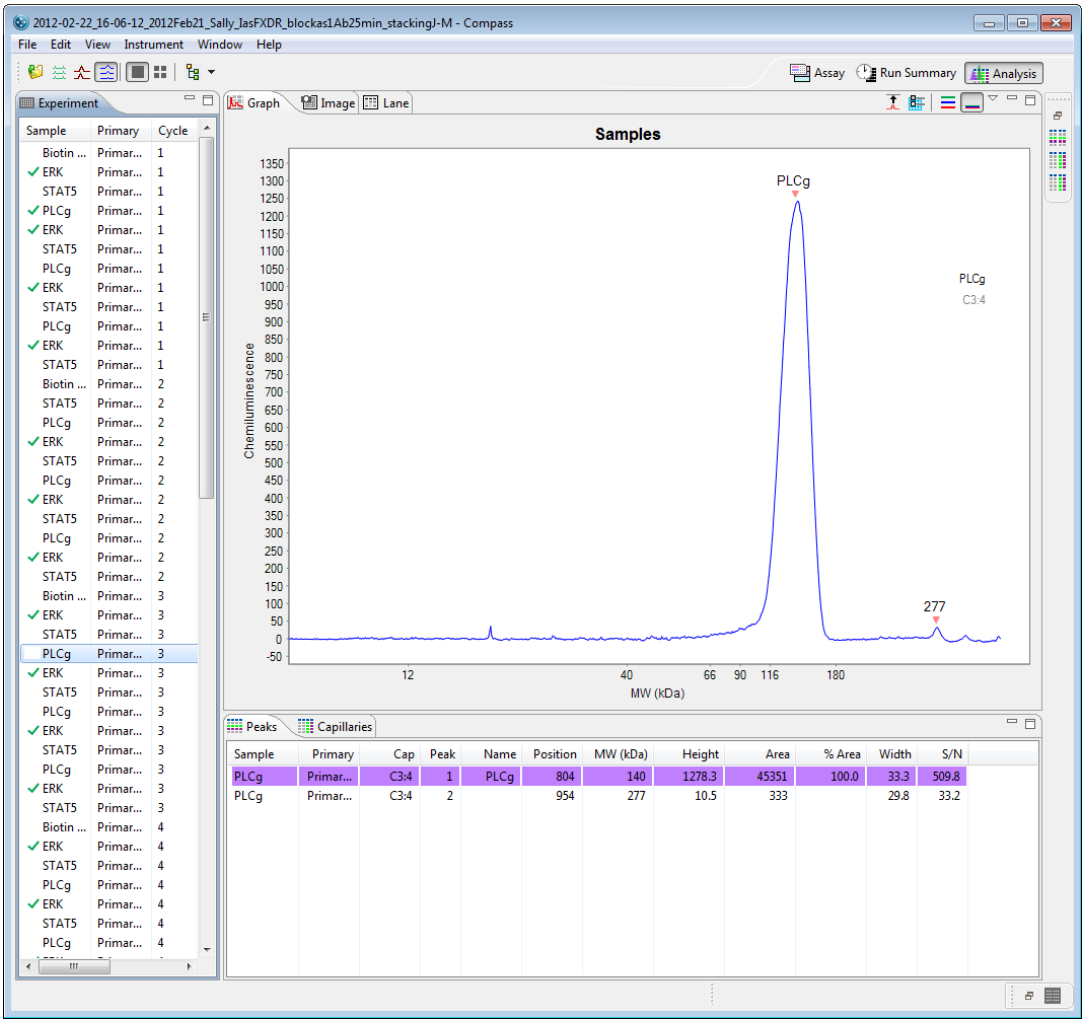


6. If you need to change the peak names group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.

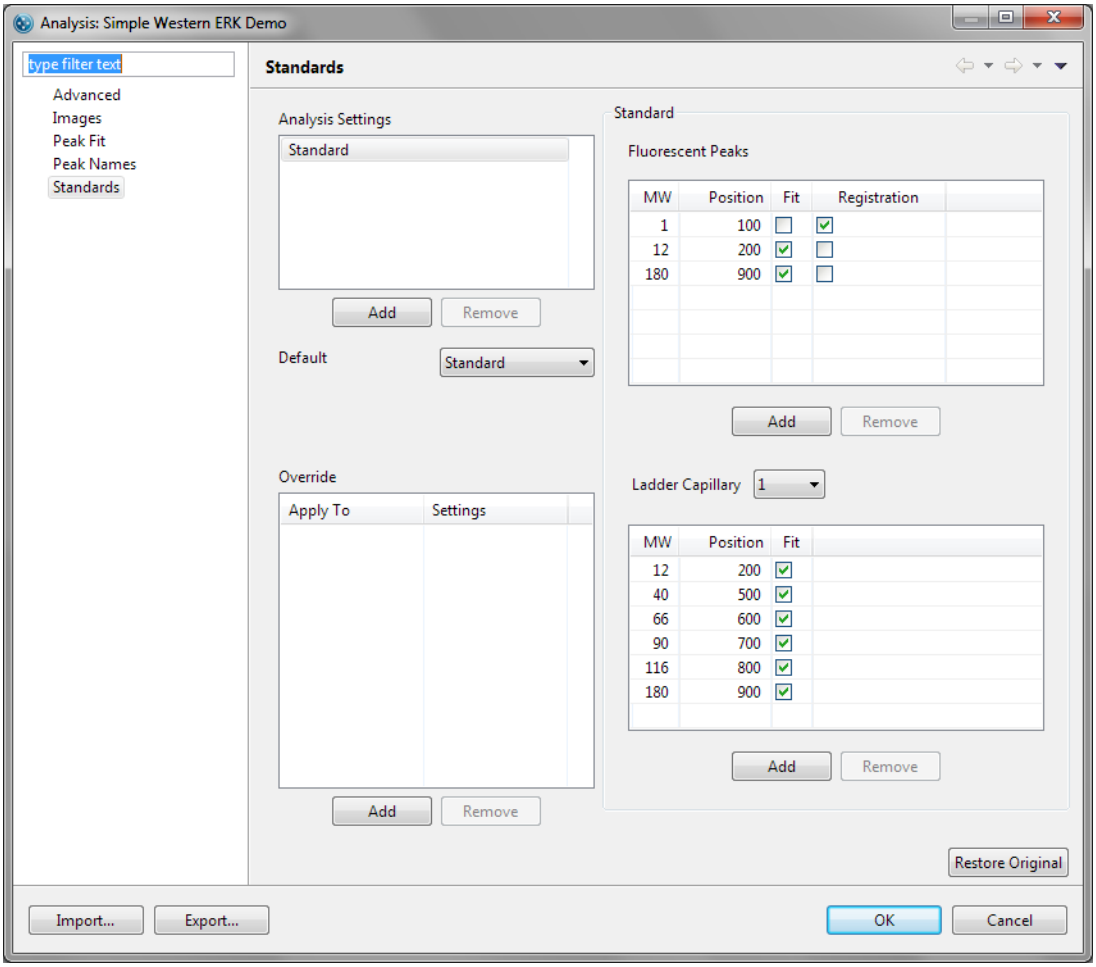
9. Click **OK** to save changes. Named peaks will be identified with a peak name label in the electropherogram and virtual blot, and will be color coded in the peaks and capillaries tables:



Standards Settings

The standards analysis settings page lets you view and change the molecular weight and position for ladder and fluorescent standards, set the registration peak, and change the capillary used for the ladder. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 261.

- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 261.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

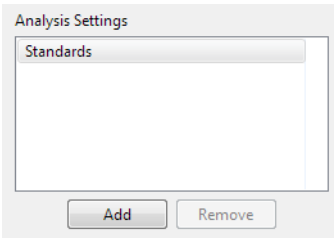
Standards Analysis Settings Groups

Standards settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
ProteinSimple recommends using the Compass default values for standards analysis settings. These settings are included in the default Standards group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 261.

Standards groups are displayed in the analysis settings box:



The Standards group shown contains the Compass default settings. Users can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Changing the Capillary Used for the Ladder

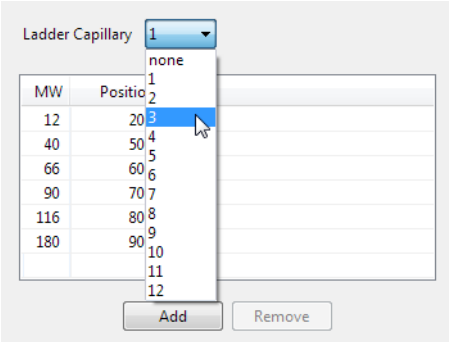
Known ladder standards are used to calculate the molecular weights of unknown sample proteins. Capillary 1 is typically used for ladder. However, users can change the ladder capillary as needed, or opt to not use a ladder at all.

NOTES:
When the ladder capillary is set to none, fluorescent standards information is used to calculate sample protein molecular weight instead of the ladder.

The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

To change the ladder capillary:

1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click the arrow in the drop down list next to Ladder Capillary, then click a capillary number or none from the list.

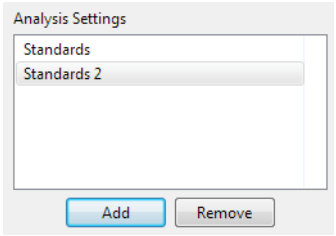


Compass will use the data in the selected capillary to recalculate molecular weights for sample proteins in the run data using the information in the ladder table. If none is selected, Compass will instead use the information in the fluorescent peaks table (fluorescent standards) to calculate molecular weight for sample proteins.

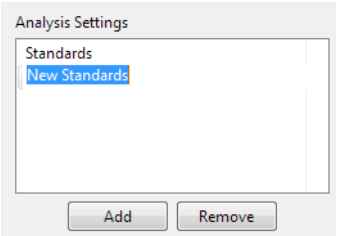
NOTE: When the ladder capillary is set to none, the ladder table becomes inactive and cannot be modified.

Creating a New Standards Group

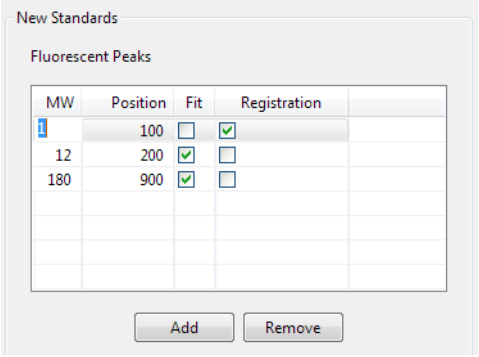
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



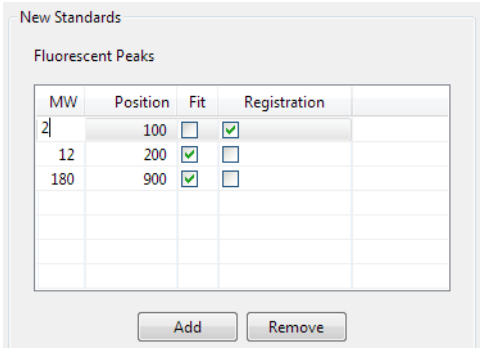
3. Click on the new group and enter a new name.



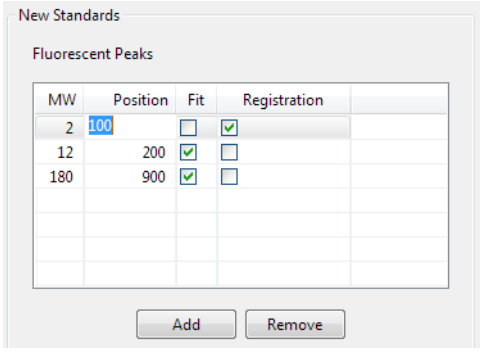
4. Click in the first cell in the **MW** column in the Fluorescent Peaks table.



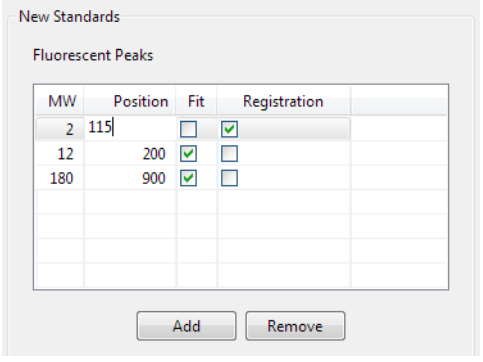
5. Enter the molecular weight (in kDa) for the fluorescent standard.



6. Click in the first cell in the **Position** column.

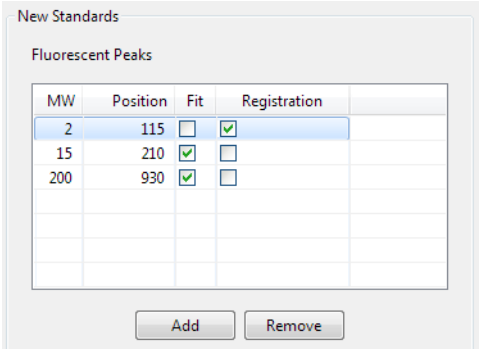


7. Enter the position of the fluorescent standard peak.



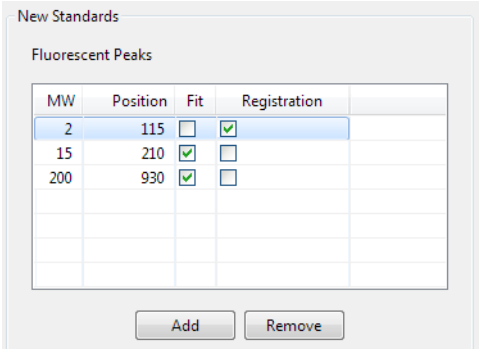
NOTE: Standards peak positions are relative to each other. Only the difference in their position is used to help identify the standard peaks. When entering standard peak information for the first time, review the standards data in the Analysis screen to find the correct peak position.

8. Repeat the steps above for the remaining standards in the table.
   - **To add another standard** - Click **Add** under the peak table, then modify the information in the new row.
   - **To remove a standard** - Select its row and click **Remove**.
9. Select which standard should be used for capillary registration by clicking the checkbox in the **Registration** column. The first standard is typically used for the registration.

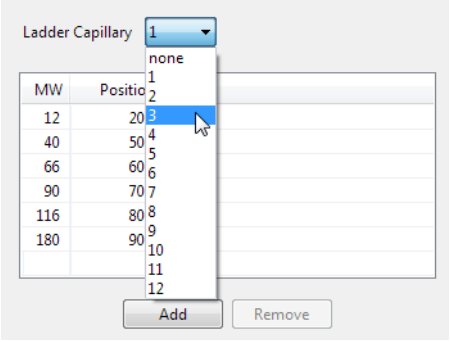


NOTE: In order for Compass to perform data analysis, at least one peak must be selected for registration.

10. Select which standards should be used for molecular weight determination of sample proteins by clicking the checkbox in the **Fit** column. The standards not used for registration are typically used for fit.



11. Click the arrow in the drop down list next to Ladder Capillary, then click a capillary number or none from the list. Capillary 1 is typically used for the ladder.



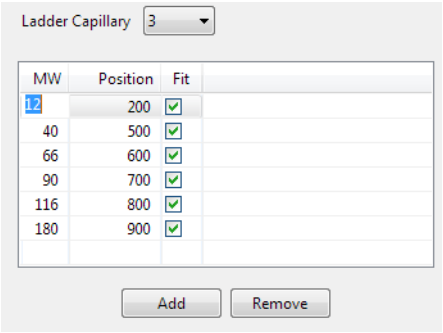
Compass will use the data in the selected capillary to calculate the molecular weights for sample proteins using the information in the ladder table. If none is selected, Compass will instead use the information in the fluorescent peaks table (fluorescent standards) to calculate molecular weight for sample proteins.

NOTES:
When the ladder capillary is set to none, the ladder table becomes inactive and cannot be modified.

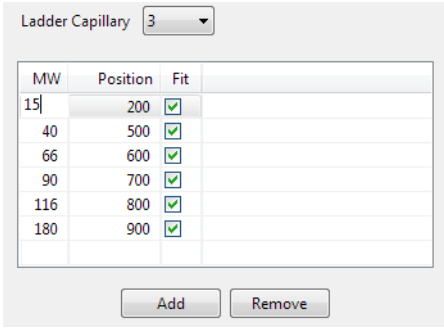
The reported molecular weight for immunodetected sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

---

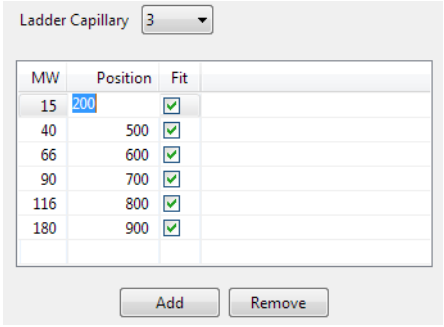
12. If a ladder capillary was selected, click in the first cell in the **MW** column in the ladder table.



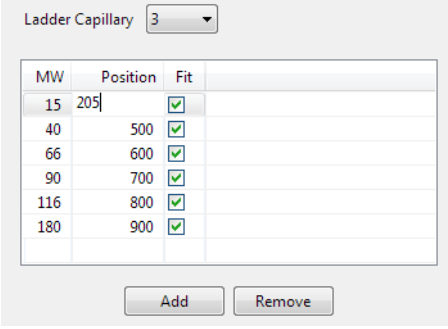
Enter the molecular weight (in kDa) for the ladder standard.



13. Click in the first cell in the **Position** column.



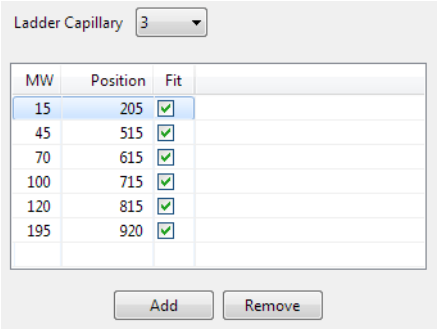
Enter the position of the ladder standard peak.



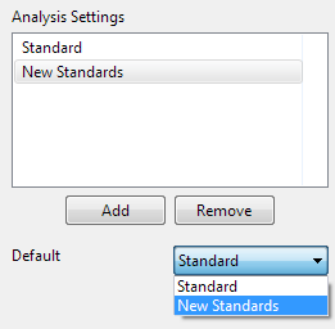
NOTE:
Ladder peak positions are relative to each other. Only the difference in their position is used to help identify the ladder peaks. When entering ladder peak information for the first time, review the ladder data in the Analysis screen to find the correct peak position.

---

14. Repeat the steps above for the remaining ladder standards in the table.
    - **To add another ladder standard** - Click **Add** under the table, then modify the information in the new row.
    - **To remove a ladder standard** - Select its row and click **Remove**.
15. Select which ladder standards should be used for molecular weight determination of sample proteins by clicking the checkbox in the **Fit** column. All ladder standards are typically used for fit.



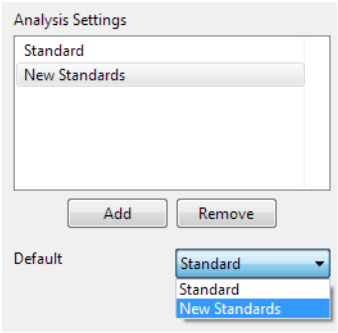
16. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.



17. Click **OK** to save changes.

Changing the Default Standards Group

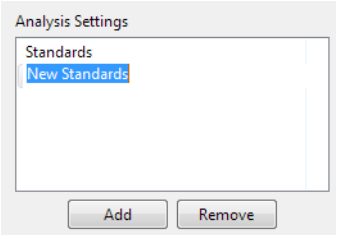
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying a Standards Group

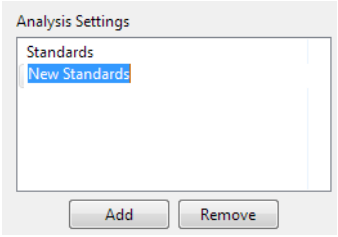
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify fluorescent standards and ladder standards information as described in "Creating a New Standards Group" on page 252.
4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

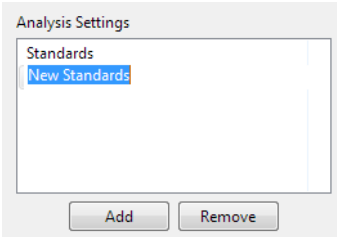
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



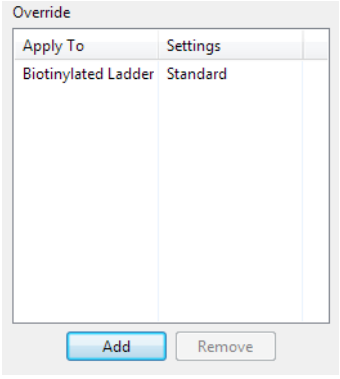
3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

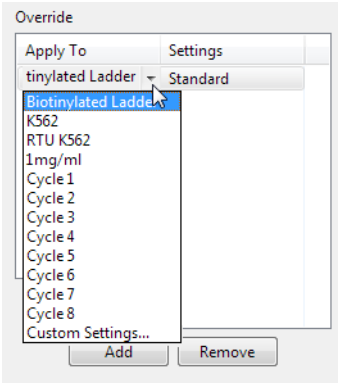
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

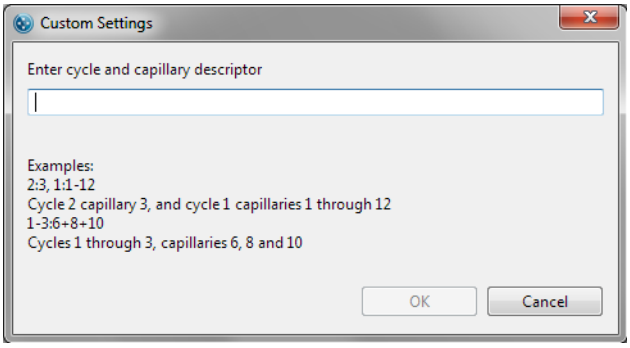


4. Click the cell in the **Apply To** column, then click the down arrow.

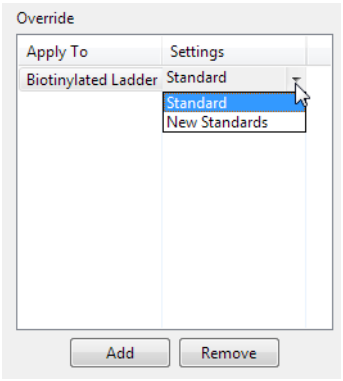


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Importing and Exporting Analysis Settings

The analysis settings in a run file can be exported as a separate file. This allows the same analysis settings to be imported into other assays or run files at a later time, rather than having to re-enter settings manually.

Importing Analysis Settings

NOTE: Importing an analysis settings file populates the settings in all Analysis pages.

---

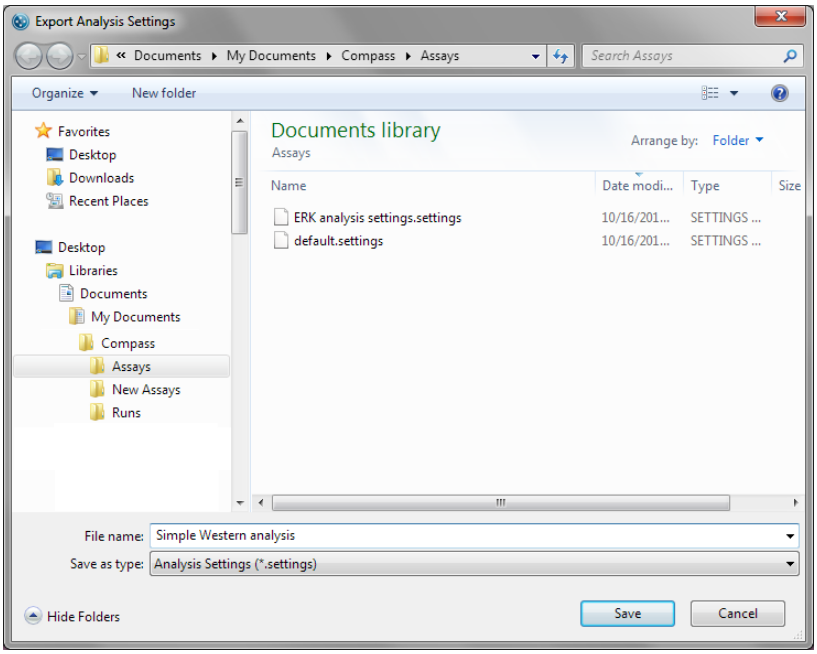
1. Open the run file or assay you want to import analysis settings to.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).
3. Click **Import** on any page.
4. Select a settings file (*.settings) and click **OK**. The imported settings will display in all analysis pages.

Exporting Analysis Settings

NOTE: Exporting an analysis settings file exports the settings in all Analysis pages.

---

1. Open the run file or assay you want to export analysis settings from.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).
3. Click **Export** on any page. The following window displays:



4. The default directory is Compass/Assays. Change the directory if needed.
5. Enter a file name and click **Save**. The settings will be saved as a *.settings file.

Chapter 9:

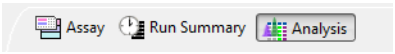
Charge Assay Data Analysis

Chapter Overview

- Analysis Screen Overview
- Opening Run Files
- How Run Data is Displayed in the Analysis Screen
- Viewing Run Data
- Compass Run Data Notifications and Warnings
- Checking Your Results
- Group Statistics
- Copying Data Views and Results Tables
- Exporting Run Files
- Changing Sample Protein Identification
- Changing the Virtual Blot View
- Changing the Electropherogram View
- Closing Run Files
- Compass Analysis Settings Overview
- Advanced Analysis Settings
- Images Analysis Settings
- Peak Fit Analysis Settings
- Peak Names Settings
- Standards Settings
- Importing and Exporting Analysis Settings

Analysis Screen Overview

The Analysis screen is used to view run data including electropherograms, capillary images, lane view data and tabulated results. Any post-run analysis is also done in this screen. To access this screen, click **Analysis** in the screen tab:



Analysis Screen Panes

The Analysis screen has six panes, each displays the following data for up to 96 capillaries per experimental run:

- **Experiment** - Lists the assay protocol steps and assay template information.
- **Graph** - Displays electropherogram data for immunodetected sample proteins, fluorescent standards or capillary registrations.
- **Image** - Displays a 12-capillary image of the separated immunodetected sample proteins, fluorescent standards or capillary registrations.
- **Lane** - Displays data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results.
- **Peaks** - Lists the tabulated results for immunodetected sample proteins and information for identified fluorescent standards and capillary registrations.
- **Capillaries** - Displays a list of the immunodetected sample proteins Compass named automatically using the user-defined peak name analysis parameters.

Sample	Primary	Cycle
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1

Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
High pho...	ERK1/2	C1:1	1	ppErk1	499	5.468	394.8	6337	58.7	0.0464	517.9
High pho...	ERK1/2	C1:1	2	pErk1	583	5.735	153.9	1411	13.1	0.0265	321.1
High pho...	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7
High pho...	ERK1/2	C1:1	4	Erk1	639	5.904	401.0	3054	28.3	0.0220	879.5
High pho...	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2
High pho...	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9

NOTE: The reported pI for immunodetected sample proteins in Compass may vary slightly from predicted pIs based on sample, buffer and assay conditions.

Software Menus Active in the Analysis Screen

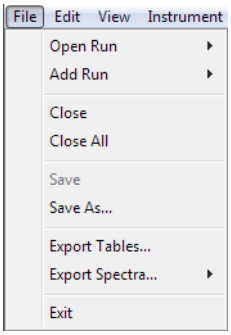
The following software menus are available:

- File
- Edit
- View
- Instrument (when Compass is connected to Peggy)
- Window
- Help

The File, Edit and View menu options specific to the Analysis screen are described next.

File Menu

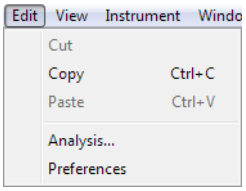
The following File menu options are active:



- **Open Run** - Opens a run file.
- **Add Run** - Opens and views other run files in addition to those that are already open.
- **Close** - Closes the run file currently being viewed.
- **Close All** - Closes all open run files.
- **Save** - Saves changes to the open run file.
- **Save As** - Saves changes to the open run file under a different file name.
- **Export Tables** - Exports the results for all capillaries in the run in .txt format.
- **Export Spectra** - Exports the raw data traces for each capillary in the run in .txt or .cdf format.
- **Exit** - Closes Compass.

Edit Menu

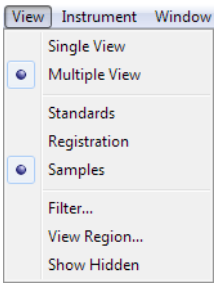
The following Edit menu options are active:



- **Copy** - Lets you copy data shown in the graph, lane, peaks or capillaries panes. See "Copying Data Views and Results Tables" on page 310 for more information.
- **Analysis** - Displays the analysis settings used to analyze the run data and lets you change them as needed. See "Compass Analysis Settings Overview" on page 348 for more information.
- **Preferences** - Lets you set and save custom preferences for data export, plot colors in the graph and Peggy's Twitter settings. See Chapter 10, *"Setting Custom User Preferences"* for more information.

View Menu

The following View menu options are active:



- **Single View** - Displays data in a per capillary (single) view format.
- **Multiple View** - Displays data in a per 12-capillary (multiple) view format.
- **Standards** - Lets you change the data view to show only the fluorescent standards.
- **Registration** - Lets you change the data view to show only the capillary registrations.
- **Samples** - Lets you change the data view to show only immunodetected sample proteins.
- **Filter** - Lets you display data only for specific capillaries or named proteins.
- **View Region** - Lets you change the pI (x-axis) range of the data displayed.
- **Show Hidden**- Shows capillaries that are hidden from the data view.

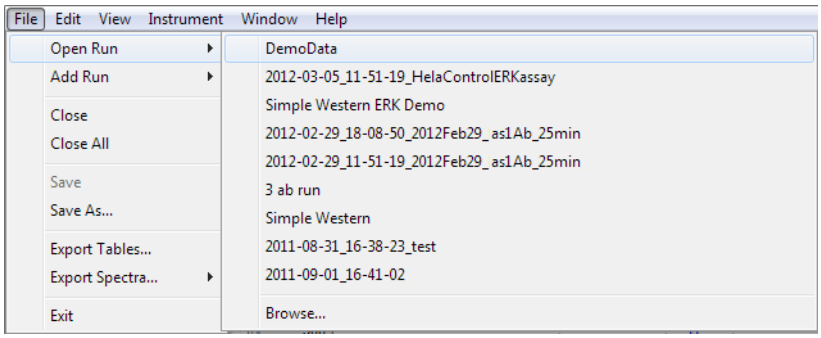
Opening Run Files

You can open one or multiple run files at a time to compare data between runs.

Opening One Run File

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

1. Select **File** in the main menu and click **Open Run**.

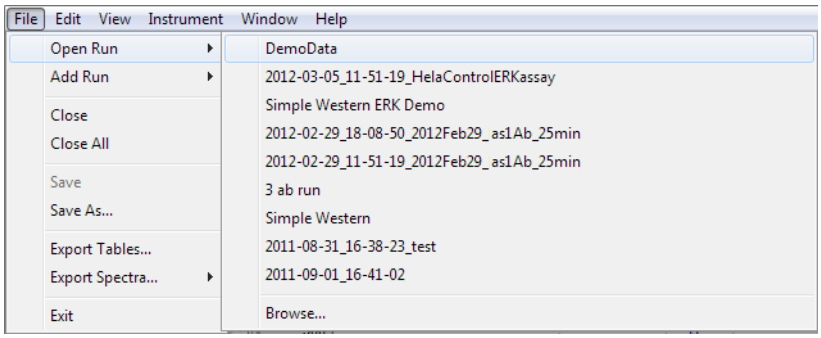


2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

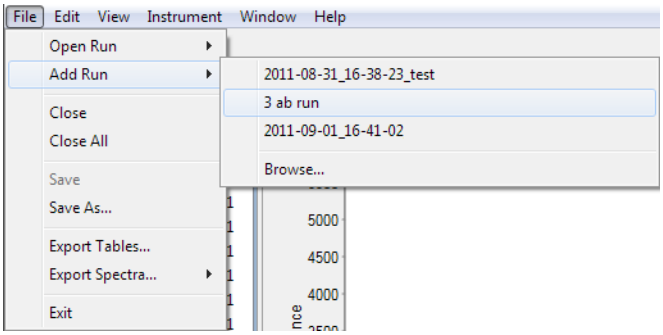
Opening Multiple Run Files

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

1. To open the first run file, select **File** in the main menu and click **Open Run**.



2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
3. To open another run file, select **File** in the main menu and click **Add Run**.



4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file. When a run is added, its data will append to the open run file, and will display as a second set of up to 96 capillaries in all screen panes. The second run file name will also appear in the Compass title bar:

Sample	Primary	Cycle
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1

Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
High pho...	ERK1/2	C1:1	1	ppErk1	499	5.468	394.8	6337	58.7	0.0464	517.9
High pho...	ERK1/2	C1:1	2	pErk1	583	5.735	153.9	1411	13.1	0.0265	321.1
High pho...	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7
High pho...	ERK1/2	C1:1	4	Erk1	639	5.904	401.0	3054	28.3	0.0220	879.5
High pho...	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2
High pho...	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9

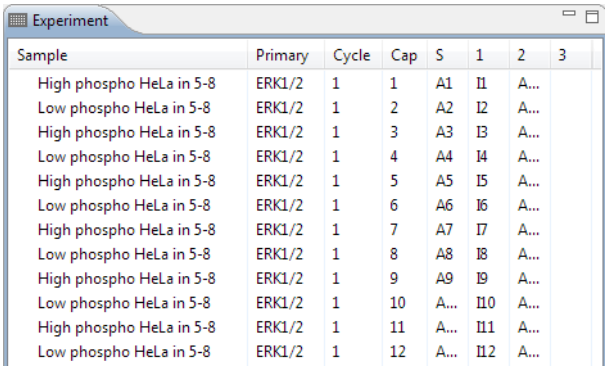
5. Repeat the last two steps to open additional runs.

How Run Data is Displayed in the Analysis Screen

Data in the run file is compartmentalized for easy review in one of six panes in the Analysis screen.

Experiment Pane: Assay and Capillary Information

The experiment pane displays assay and capillary information for each of the 96 capillaries in the run. A maximized view of the experiment pane is shown below.


Experiment

Sample	Primary	Cycle	Cap	S	1	2	3
High phospho HeLa in 5-8	ERK1/2	1	1	A1	I1	A...	
Low phospho HeLa in 5-8	ERK1/2	1	2	A2	I2	A...	
High phospho HeLa in 5-8	ERK1/2	1	3	A3	I3	A...	
Low phospho HeLa in 5-8	ERK1/2	1	4	A4	I4	A...	
High phospho HeLa in 5-8	ERK1/2	1	5	A5	I5	A...	
Low phospho HeLa in 5-8	ERK1/2	1	6	A6	I6	A...	
High phospho HeLa in 5-8	ERK1/2	1	7	A7	I7	A...	
Low phospho HeLa in 5-8	ERK1/2	1	8	A8	I8	A...	
High phospho HeLa in 5-8	ERK1/2	1	9	A9	I9	A...	
Low phospho HeLa in 5-8	ERK1/2	1	10	A...	I10	A...	
High phospho HeLa in 5-8	ERK1/2	1	11	A...	I11	A...	
Low phospho HeLa in 5-8	ERK1/2	1	12	A...	I12	A...	

- **To view all columns** - Click the **Experiment** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Experiment** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Experiment pane column descriptions for the Peggy Charge default assay are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.

NOTE: Data notification icons will display in the sample column if Compass detected a potential analysis issue or data was manually modified by the user. For more information see "Compass Run Data Notifications and Warnings" on page 294.

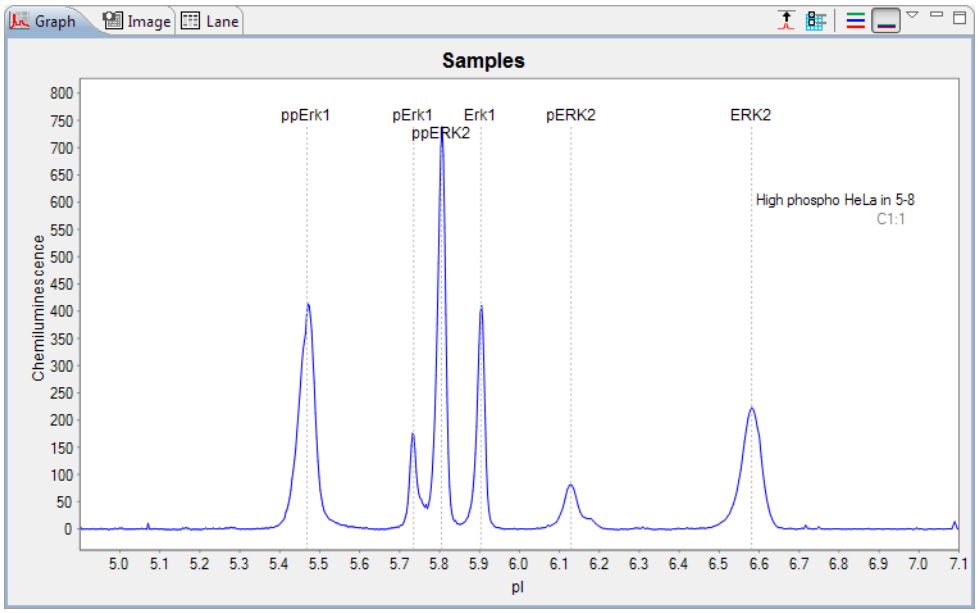
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Cycle** - Run cycle number. There are 12 capillaries in one cycle.

NOTE: Peggy can run up to eight cycles (12 capillaries per cycle) in an experiment. Each cycle is designated in the Experiment summary.

- **Cap** - Capillary number.
- **S** - Well on the assay plate used for sample.
- **1** - Well on the assay plate used for primary antibody.
- **2** - Well on the assay plate used for secondary antibody.
- **3** - Well on the assay plate used for tertiary antibody (if used).

Graph Pane: Electropherogram Data

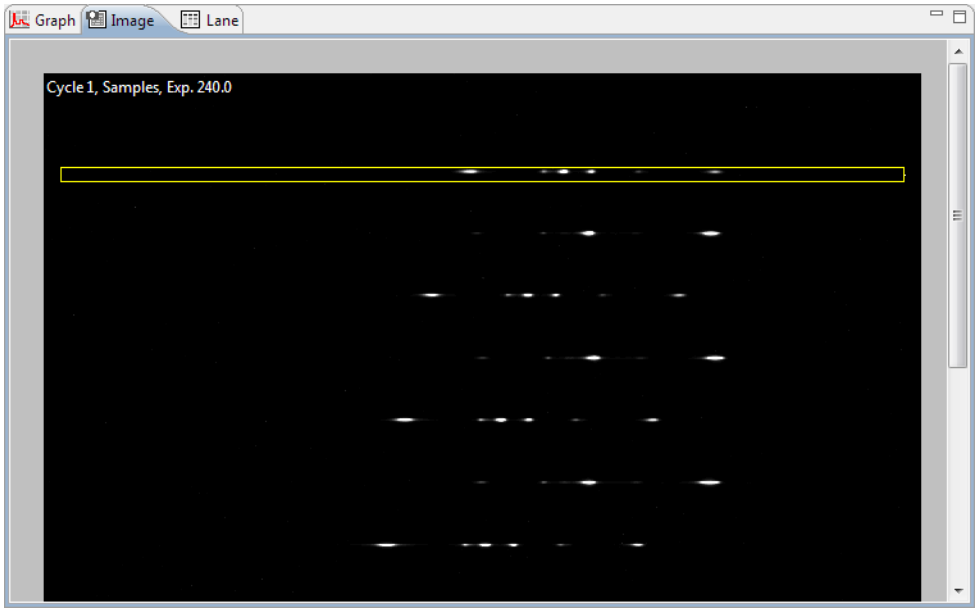
Click the **Graph** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations. Data for samples is shown in the following example, and immunodetected proteins are displayed as peaks:



More Graph view options will be described in more detail in "Changing the Electropherogram View" on page 325.

Image Pane: Capillary Separation Image Data

Click the **Image** tab to view final separation images of immunodetected sample proteins, fluorescent standards or capillary registrations for up to 96 capillaries per experimental run. Image data for samples is shown in the following example:



Lane Pane: Virtual Blot-Like Image Data

Click the **Lane** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results. Data for samples in the lane view is shown in the following example, and immunodetected proteins are displayed as bands.

Graph
Image
Lane
C1:1 : High phospho HeLa in 5-8
C1:2 : Low phospho HeLa in 5-8
C1:3 : High phospho HeLa in 5-8
C1:4 : Low phospho HeLa in 5-8
C1:5 : High phospho HeLa in 5-8
C1:6 : Low phospho HeLa in 5-8
C1:7 : High phospho HeLa in 5-8
C1:8 : Low phospho HeLa in 5-8
C1:9 : High phospho HeLa in 5-8
C1:10 : Low phospho HeLa in 5-8
C1:11 : High phospho HeLa in 5-8
C1:12 : Low phospho HeLa in 5-8
Erk1
pI: 5.90
Area: 13618

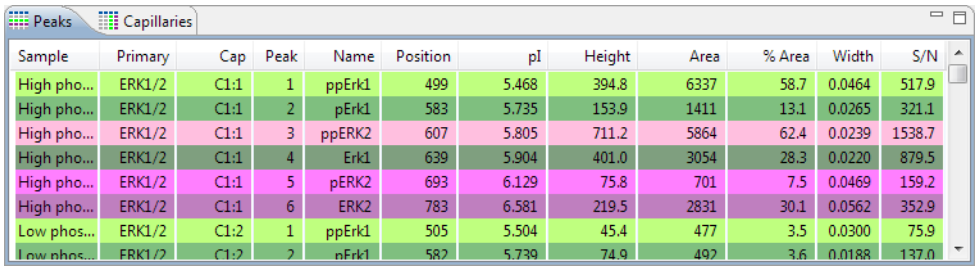
To view information for a band, roll the mouse over a band until the info box appears.

NOTE: The reported pI for immunodetected sample proteins in Compass may vary slightly from predicted pIs based on sample, buffer and assay conditions.

Lane data displayed in the virtual blot is automatically aligned by Compass. To view raw, unaligned lane data and learn more about virtual blot viewing options, see "Changing the Virtual Blot View" on page 319.

Peaks Pane: Calculated Results

Click the **Peaks** tab to view tabulated results for immunodetected sample proteins, fluorescent standards or capillary registrations. Each row in the table shows the individual results for each peak detected in each capillary. Data for samples in the peaks table is shown in the following example:



Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
High pho...	ERK1/2	C1:1	1	ppErk1	499	5.468	394.8	6337	58.7	0.0464	517.9
High pho...	ERK1/2	C1:1	2	pErk1	583	5.735	153.9	1411	13.1	0.0265	321.1
High pho...	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7
High pho...	ERK1/2	C1:1	4	Erk1	639	5.904	401.0	3054	28.3	0.0220	879.5
High pho...	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2
High pho...	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9
Low phos...	ERK1/2	C1:2	1	ppErk1	505	5.504	45.4	477	3.5	0.0300	75.9
Low phos...	ERK1/2	C1:2	2	pErk1	582	5.739	74.9	492	3.6	0.0188	137.0

NOTES:
Peaks that Compass names automatically using the user-defined peak name analysis parameters are color-coded.

The reported pI for immunodetected sample proteins in Compass may vary slightly from predicted pIs based on sample, buffer and assay conditions.

- **To view all rows** - Click the **Peaks** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Peaks** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Peak table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Cap** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

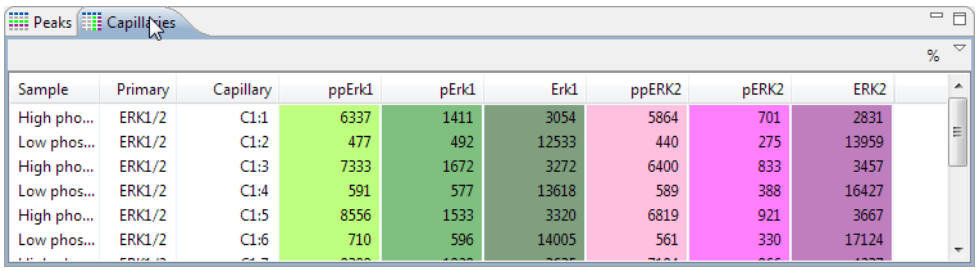
NOTE: Peggy runs up to eight cycles in an experiment, 12 capillaries at a time. Capillary and cycle numbers are displayed in the Experiment tab.

- **Peak** - Peak number. Peaks are numbered in order of detection.

- **Name** - Peak name. Displays peaks that Compass named automatically using the user-defined peak name analysis parameters. Cells will be blank if Compass was not able to name the peak or if naming parameters were not entered.
- **Position** - Displays the pixel position of a peak in the image.
- **pI** - Displays the calculated pI for the peak.
- **Height** - Displays the calculated peak height.
- **Area** - Displays the calculated peak area.
- **% Area** - Displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100 (shown for named peak sample data only).
- **Width** - Displays the calculated peak width (shown for sample data only).
- **S/N** - Displays the calculated signal to noise ratio for the peak.

Capillaries Pane: User-Specified Peak Names

Click the **Capillaries** tab to view tabulated results for sample proteins associated with specific primary antibodies in the run data. Compass labels these sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary. Data for samples in the capillaries table is shown in the following example.



Sample	Primary	Capillary	ppErk1	pErk1	Erk1	ppERK2	pERK2	ERK2
High pho...	ERK1/2	C1:1	6337	1411	3054	5864	701	2831
Low phos...	ERK1/2	C1:2	477	492	12533	440	275	13959
High pho...	ERK1/2	C1:3	7333	1672	3272	6400	833	3457
Low phos...	ERK1/2	C1:4	591	577	13618	589	388	16427
High pho...	ERK1/2	C1:5	8556	1533	3320	6819	921	3667
Low phos...	ERK1/2	C1:6	710	596	14005	561	330	17124

NOTES:
Peaks that Compass names automatically with user-defined peak name settings are color-coded.

Information displayed for fluorescent standards and capillary registration data will be for identified standards or registration peaks.

- **To view all rows** - Click the **Capillaries** tab, then use the scroll bar or click **Maximize** in the upper right corner.

- **To resize columns** - Click the **Capillaries** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Capillaries table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

NOTE: Peggy runs12 capillaries at a time in a cycle and is able to run up to eight cycles in an experiment. The Information on cycle and capillary number is displayed in the Experiment tab.

- **Peak Name Columns** - An individual column per peak name will display for every peak identified by name in the run data. Cells for capillaries in these columns will be blank if Compass did not find peaks automatically using the user-defined peak name analysis parameters (or none were entered).
  - **To view % area in the peak name columns** - Select **%** in the upper right corner of the pane. This displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100.

Sample	Primary	Capillary	ppErk1	pErk1	Erk1	ppERK2	pERK2	ERK2
High pho...	ERK1/2	C1:1	58.7	13.1	28.3	62.4	7.5	30.1
Low phos...	ERK1/2	C1:2	3.5	3.6	92.8	3.0	1.9	95.1
High pho...	ERK1/2	C1:3	59.7	13.6	26.7	59.9	7.8	32.3
Low phos...	ERK1/2	C1:4	4.0	3.9	92.1	3.4	2.2	94.4
High pho...	ERK1/2	C1:5	63.8	11.4	24.8	59.8	8.1	32.1
Low phos...	ERK1/2	C1:6	4.6	3.9	91.5	3.1	1.8	95.0

  - **To view peak area in the peak name columns (default)** - Deselect **%** in the upper right corner of the pane. This displays calculated peak area for the individual peak only.

Viewing Run Data

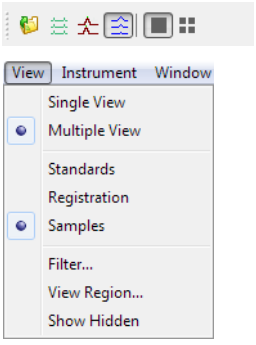
Each run file contains the following data for up to 96 capillaries:

- **Sample data** - For the immunodetected proteins in the sample.
- **Standards data** - For the fluorescent standards run with each sample.
- **Registration data** - For tracking capillaries as they are moved for various assay steps.

Data can be viewed for one, all, or individually selected capillaries.

Switching Between Sample, Standards and Registration Data Views

You can switch between viewing sample, standards and registration data in a run file using the View bar or View menu:



Data buttons in the View bar:

Show Standards

Show Registrations

Show Samples

- **To view sample data** - Click **Show Samples** in the View bar or select **View** in the main menu and click **Samples**:

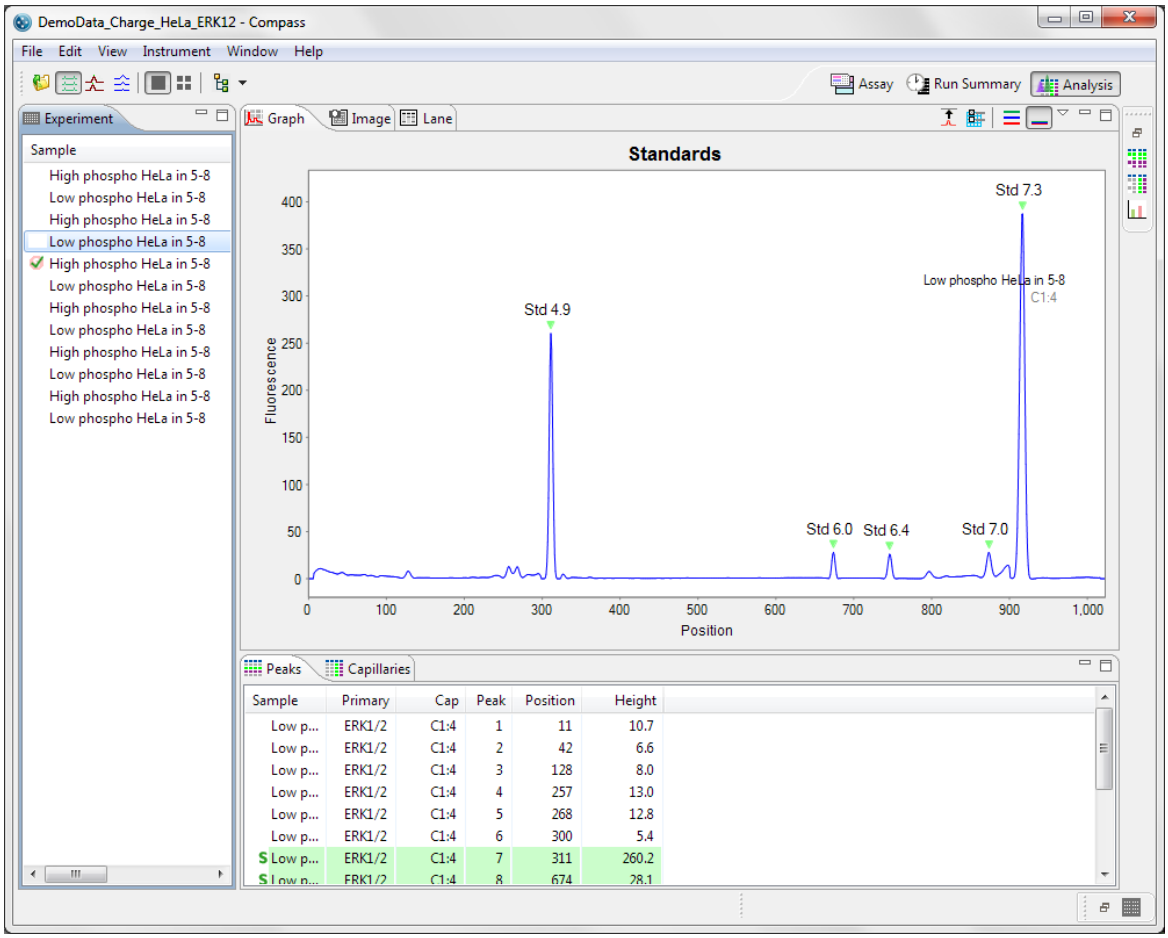
Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
High pho...	ERK1/2	C1:1	1	ppErk1	499	5.468	394.8	6337	58.7	0.0464	517.9
High pho...	ERK1/2	C1:1	2	pErk1	583	5.735	153.9	1411	13.1	0.0265	321.1
High pho...	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7
High pho...	ERK1/2	C1:1	4	Erk1	639	5.904	401.0	3054	28.3	0.0220	879.5
High pho...	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2
High pho...	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9

- Data in this view is for immunodetected sample proteins only.
- Graph view data displays electropherograms in chemiluminescence units (y-axis) and pI (x-axis).
- Lane view data displays immunodetected sample proteins only.
- Image view data displays immunodetected sample proteins only.
- Results for each immunodetected protein are shown in the peaks and capillaries tables.

NOTE: The reported pI for immunodetected sample proteins in Compass may vary slightly from predicted pIs based on sample, buffer and assay conditions.

For information on checking and identifying sample peaks, see "Step 4 – Checking Samples" on page 301.

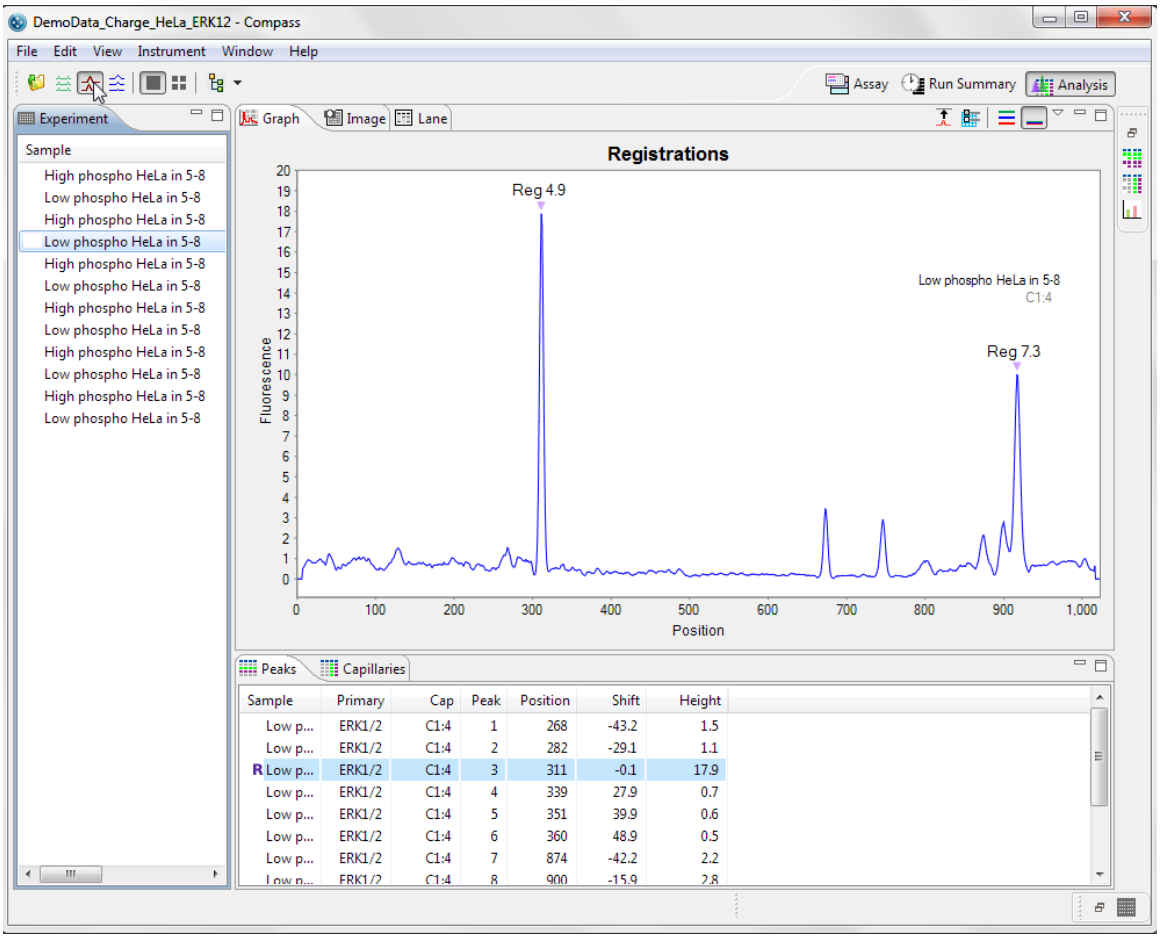
- **To view standards data** - Click **Show Standards** in the View bar or select **View** in the main menu and click **Standards**:


DemoData_Charge_HeLa_ERK12 - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Experiment
Sample
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
Graph Image Lane
Standards
Std 7.3
Low phospho HeLa in 5-8
C1:4
Std 4.9
Std 6.0 Std 6.4 Std 7.0
Fluorescence
Position
Peaks Capillaries
Sample Primary Cap Peak Position Height
Low p... ERK1/2 C1:4 1 11 10.7
Low p... ERK1/2 C1:4 2 42 6.6
Low p... ERK1/2 C1:4 3 128 8.0
Low p... ERK1/2 C1:4 4 257 13.0
Low p... ERK1/2 C1:4 5 268 12.8
Low p... ERK1/2 C1:4 6 300 5.4
S Low p... ERK1/2 C1:4 7 311 260.2
S Low p... ERK1/2 C1:4 8 674 28.1

- Data in this view is for the fluorescent standards only. These standards are premixed with each sample prior to analysis.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays fluorescent standards only.
- Image view data displays fluorescent standards only.
- Standards are highlighted in both the peaks and capillaries tables and marked with an **S**.

For information on checking and identifying standards peaks, see "Step 2 – Checking Fluorescent Sizing Standards" on page 296.

- **To view registration data** - Click **Show Registrations** in the View bar or select **View** in the main menu and click **Registration**:


DemoData_Charge_HeLa_ERK12 - Compass
File Edit View Instrument Window Help
Assay
Run Summary
Analysis
Experiment
Sample
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
Graph
Image
Lane
Registrations
Reg 4.9
Reg 7.3
Low phospho HeLa in 5-8
C1:4
Fluorescence
Position
Peaks
Capillaries
Sample Primary Cap Peak Position Shift Height
Low p... ERK1/2 C1:4 1 268 -43.2 1.5
Low p... ERK1/2 C1:4 2 282 -29.1 1.1
R Low p... ERK1/2 C1:4 3 311 -0.1 17.9
Low p... ERK1/2 C1:4 4 339 27.9 0.7
Low p... ERK1/2 C1:4 5 351 39.9 0.6
Low p... ERK1/2 C1:4 6 360 48.9 0.5
Low p... ERK1/2 C1:4 7 874 -42.2 2.2

- Data in this view shows capillary registration only.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays capillary registration only.
- Image view data displays capillary registration only.
- Registrations are highlighted in both the peaks and capillaries tables and marked with an R.

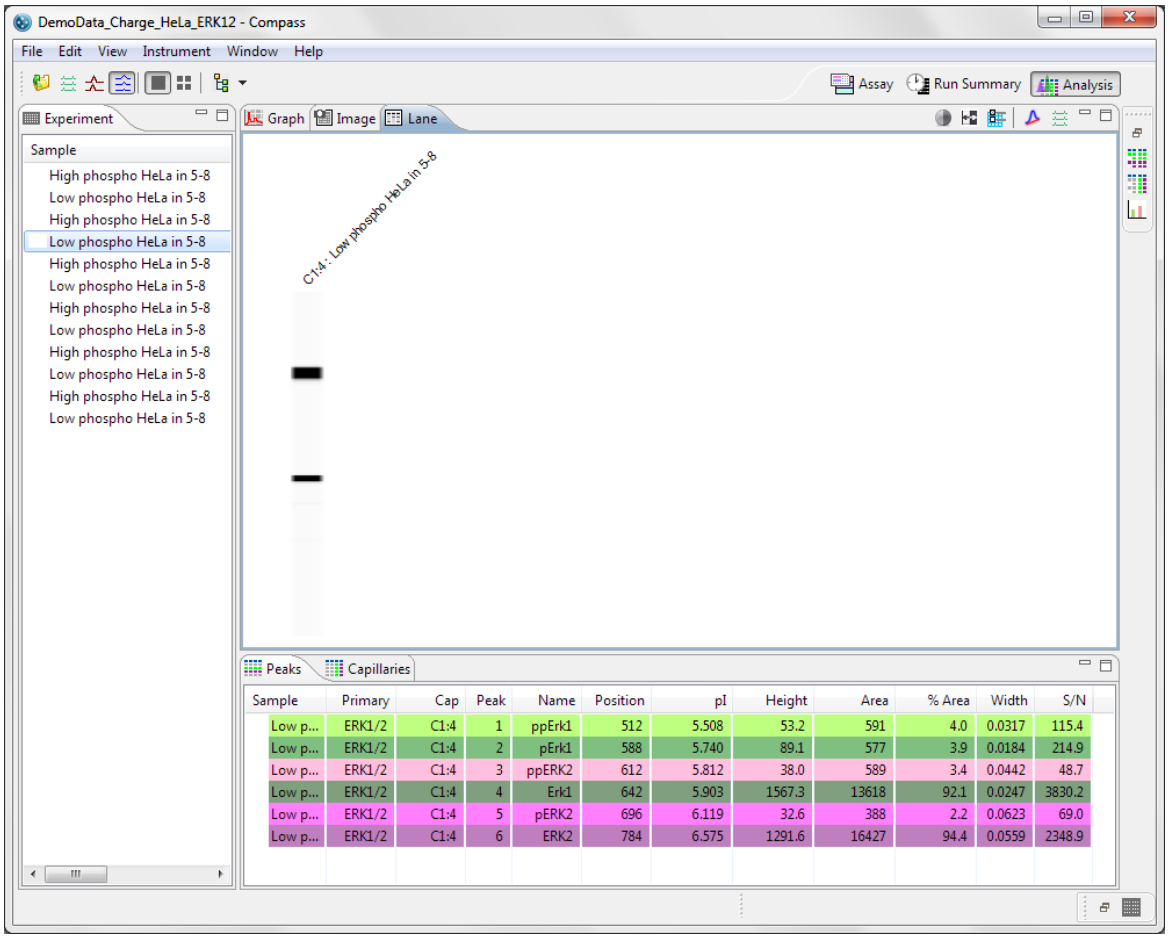
Because capillaries must be moved multiple times during the run, their positions are tracked to account for potential changes in imaging position as they are moved to and from the separation block. To do this, one of the standards is used as a capillary registration. An image of the standard used for registration is taken at the end of the separation step prior to chemiluminescent imaging. The position of this peak in the registration image is then compared to the position of the same standard peak in the standards image. The shift value listed in the peaks table is the change in pixel position between the two images. Compass data is then aligned to account for any changes in capillary position.

For information on checking and identifying registration peaks, see "Step 3 – Checking Capillary Registrations" on page 299.

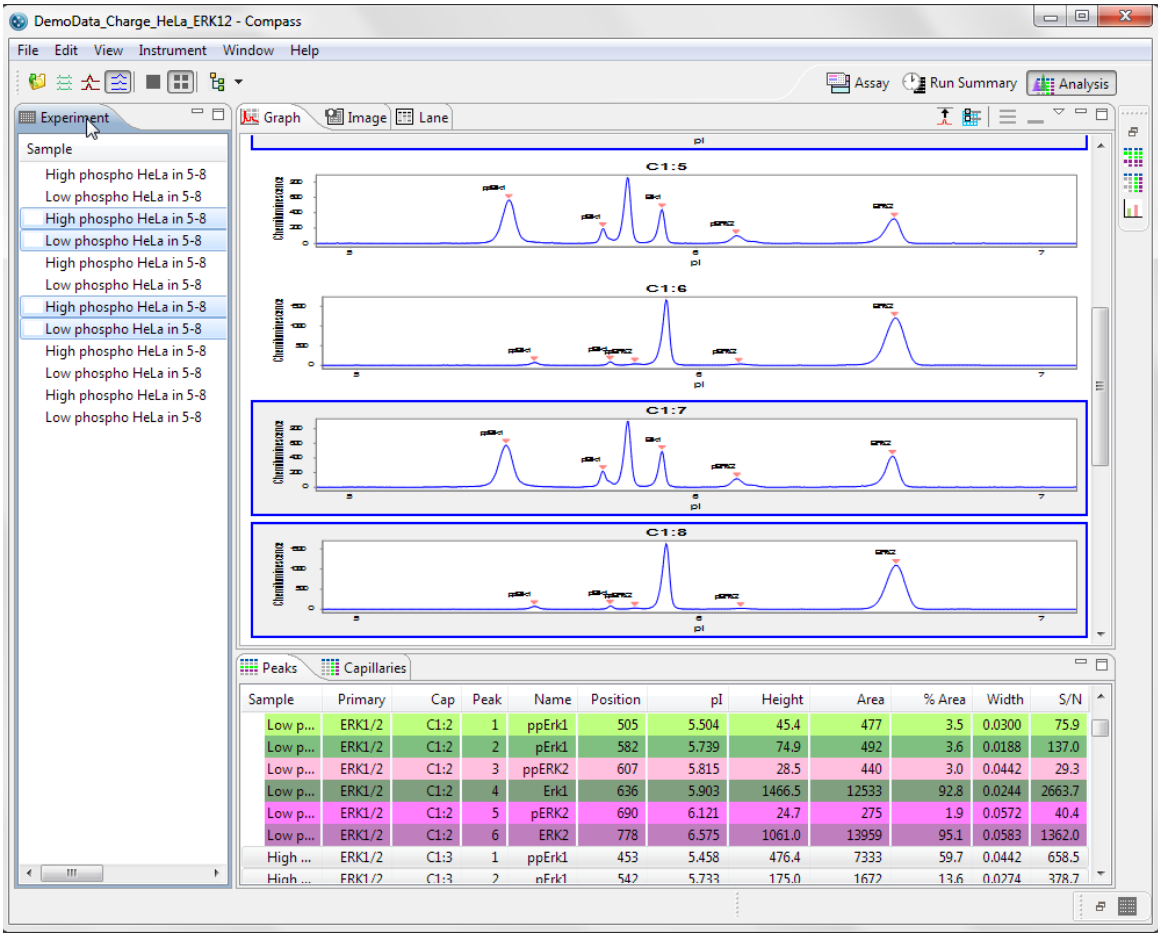
Selecting and Displaying Capillary Data

You can choose to view data from one, multiple, or all capillaries at once.

- **To look at data for one capillary** - Click a row in the experiment pane. Data for just the row selected will display in all data views and tables. The following example shows sample data displayed in the lane and peaks panes when one sample is selected:

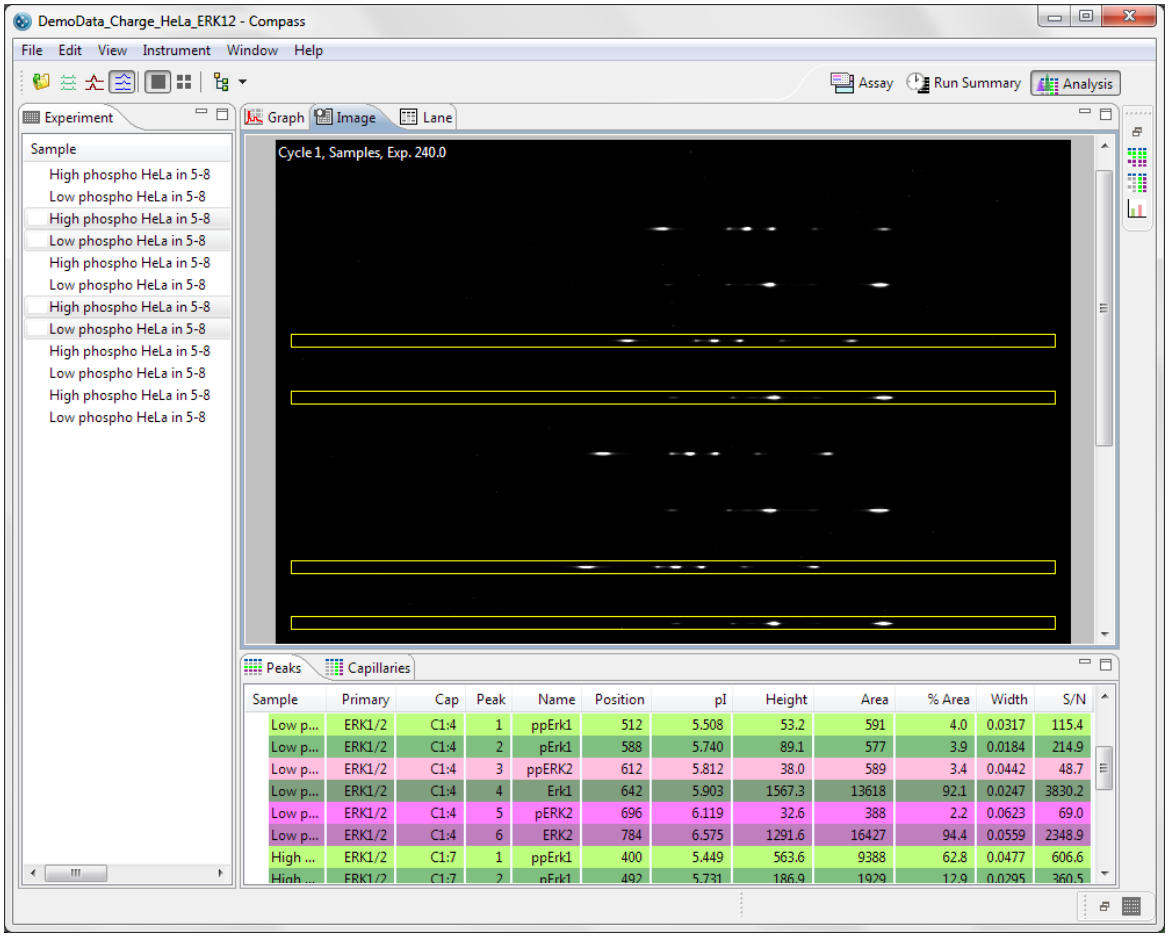

DemoData_Charge_HeLa_ERK12 - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Experiment
Sample
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
Graph Image Lane
C1:4 : Low phospho HeLa in 5-8
Peaks Capillaries
Sample Primary Cap Peak Name Position pI Height Area % Area Width S/N
Low p... ERK1/2 C1:4 1 ppErk1 512 5.508 53.2 591 4.0 0.0317 115.4
Low p... ERK1/2 C1:4 2 pErk1 588 5.740 89.1 577 3.9 0.0184 214.9
Low p... ERK1/2 C1:4 3 ppERK2 612 5.812 38.0 589 3.4 0.0442 48.7
Low p... ERK1/2 C1:4 4 Erk1 642 5.903 1567.3 13618 92.1 0.0247 3830.2
Low p... ERK1/2 C1:4 5 pERK2 696 6.119 32.6 388 2.2 0.0623 69.0
Low p... ERK1/2 C1:4 6 ERK2 784 6.575 1291.6 16427 94.4 0.0559 2348.9

- **To look at data for multiple non-sequential capillaries** - Hold the **Ctrl** key and select the rows you want to view in the experiment pane. Data for only the rows selected will display in all data views and results tables. The following example shows sample data displayed in the graph (in multiple view) and capillaries panes when multiple rows are selected:

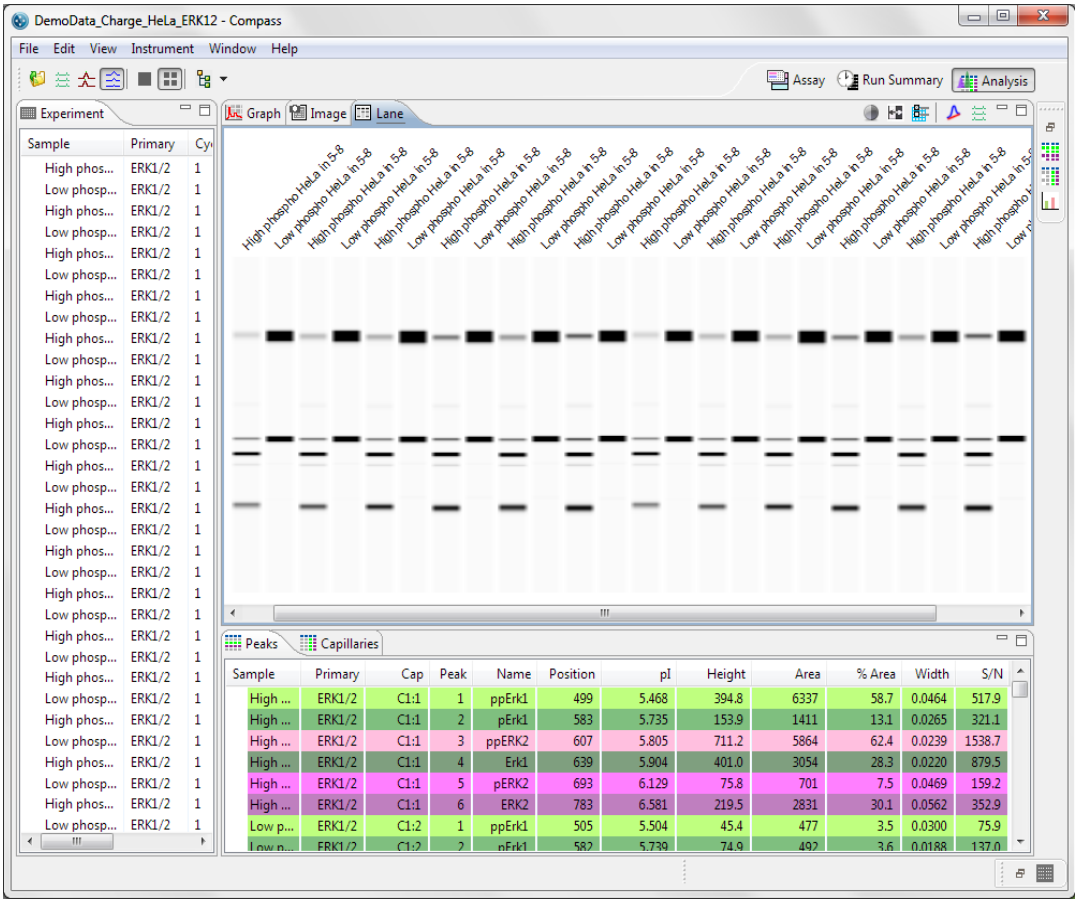


Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Low p...	ERK1/2	C1:2	1	ppErk1	505	5.504	45.4	477	3.5	0.0300	75.9
Low p...	ERK1/2	C1:2	2	pErk1	582	5.739	74.9	492	3.6	0.0188	137.0
Low p...	ERK1/2	C1:2	3	ppERK2	607	5.815	28.5	440	3.0	0.0442	29.3
Low p...	ERK1/2	C1:2	4	Erk1	636	5.903	1466.5	12533	92.8	0.0244	2663.7
Low p...	ERK1/2	C1:2	5	pERK2	690	6.121	24.7	275	1.9	0.0572	40.4
Low p...	ERK1/2	C1:2	6	ERK2	778	6.575	1061.0	13959	95.1	0.0583	1362.0
High ...	ERK1/2	C1:3	1	ppErk1	453	5.458	476.4	7333	59.7	0.0442	658.5
High ...	ERK1/2	C1:3	2	pErk1	542	5.733	175.0	1672	13.6	0.0274	378.7

- **To look at data for multiple sequential capillaries** - Hold the **Shift** key and select the first and last rows you want to view in the experiment pane. Data for only the selected rows will display in all data views and results tables. The following example shows standards data displayed in the image and peaks panes when multiple sequential rows are selected:

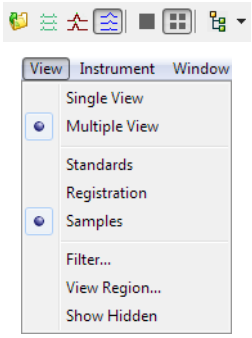

DemoData_Charge_HeLa_ERK12 - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Experiment
Sample
High phospho HeLa in 5-8
Low phospho HeLa in 5-8
Graph Image Lane
Cycle 1, Samples, Exp. 240.0
Peaks Capillaries
Sample Primary Cap Peak Name Position pI Height Area % Area Width S/N
Low p... ERK1/2 C1:4 1 ppErk1 512 5.508 53.2 591 4.0 0.0317 115.4
Low p... ERK1/2 C1:4 2 pErk1 588 5.740 89.1 577 3.9 0.0184 214.9
Low p... ERK1/2 C1:4 3 ppERK2 612 5.812 38.0 589 3.4 0.0442 48.7
Low p... ERK1/2 C1:4 4 Erk1 642 5.903 1567.3 13618 92.1 0.0247 3830.2
Low p... ERK1/2 C1:4 5 pERK2 696 6.119 32.6 388 2.2 0.0623 69.0
Low p... ERK1/2 C1:4 6 ERK2 784 6.575 1291.6 16427 94.4 0.0559 2348.9
High ... ERK1/2 C1:7 1 ppErk1 400 5.449 563.6 9388 62.8 0.0477 606.6

- **To look at data for all capillaries** - Hold the **Shift** key and select the first and last rows in the experiment pane. Data for all rows will display in all data views and results tables. The following example shows sample data displayed in the lane and peaks panes when all rows are selected. The pane scroll bars can be used to view all 8 cycles:



Switching Between Single and Multiple Views of the Capillaries

You can switch between displaying run data in the graph, image and lane panes in a per capillary (single) format or up to 96 capillary format. This view is selected in the View bar or the View menu.



Capillary view buttons in the View bar:

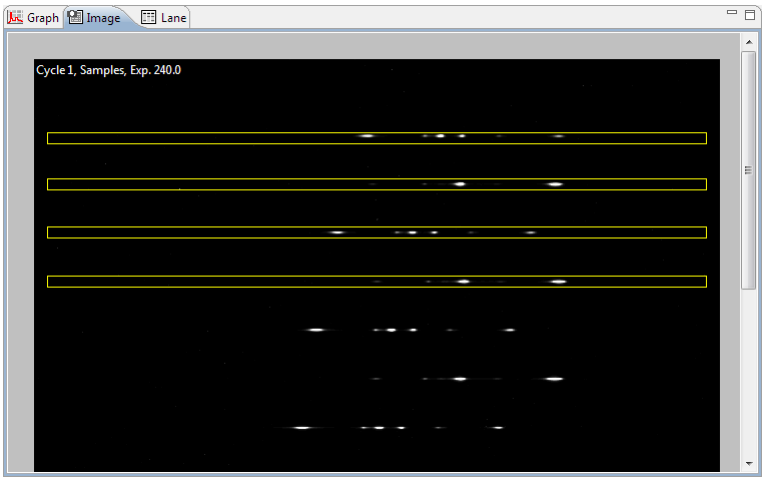
Single View

Multiple View

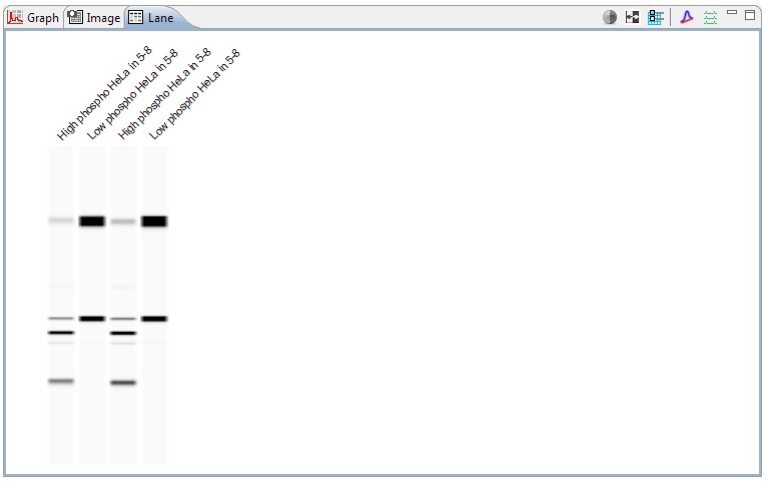
- **To view data in a per capillary format** - Click **Single View** in the View bar or select **View** in the main menu and click **Single View**:

Data for the row(s) selected in the experiment pane will display as follows:

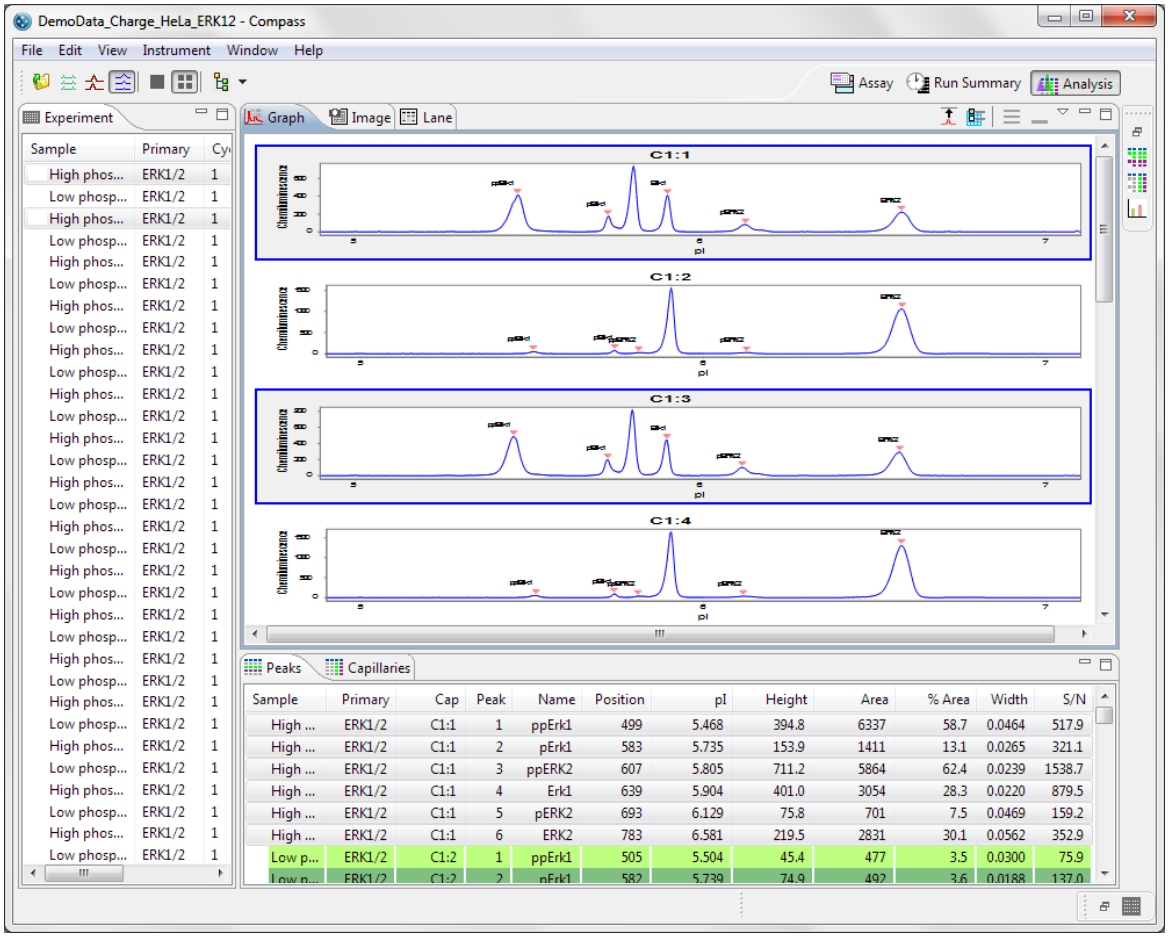
- Electropherograms in the graph pane display overlaid or stacked (depending on the graph option chosen).
- Results for only the selected row(s) will display in the peaks and capillaries tables.
- The selected row(s) are highlighted in the image pane:



- Lanes for only the selected row(s) are displayed in the lane pane:

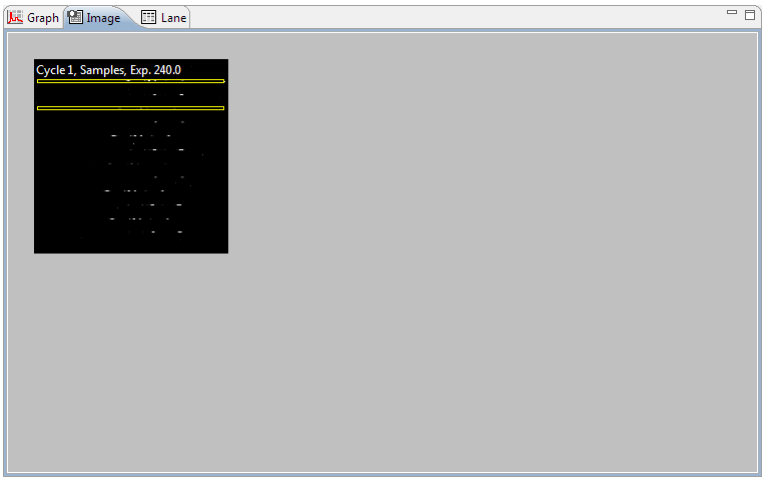


- **To view data up to 96 -capillary format** - Click **Multiple View** in the View bar or select **View** in the main menu and click **Multiple View**:

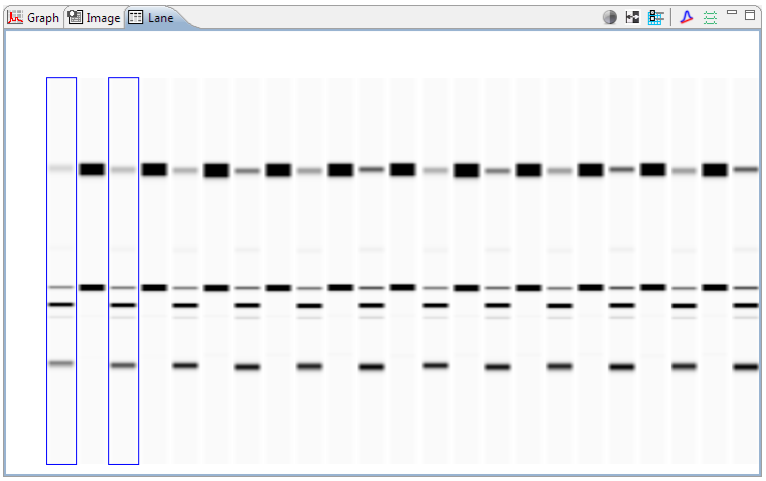


Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms corresponding to the selected row(s) are highlighted and displayed in the graph pane.
- Results for selected capillaries display in the peaks and capillaries tables and are highlighted.
- The selected row(s) are highlighted in the image pane:



- All selected lanes display in the lane pane, and lanes corresponding to the selected row(s) are highlighted.

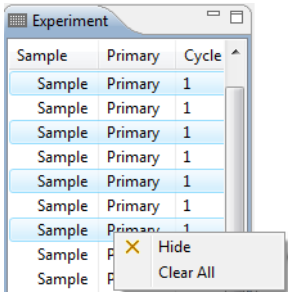


Hiding Capillary Data

Capillary data can be hidden from view if needed. To do this:

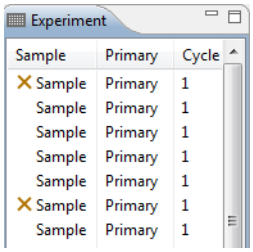
1. Click the **Experiment** tab.

2. Select the rows you want to hide, then right click on one of the selected rows and click **Hide**:



Data for selected rows will be hidden in all data views and results tables (except for the image pane).

- **To view hidden rows** - Select **View** in the main menu and click **Show Hidden**. Hidden rows will become visible again in all panes, and hidden rows will be marked with an X in the experiment pane:



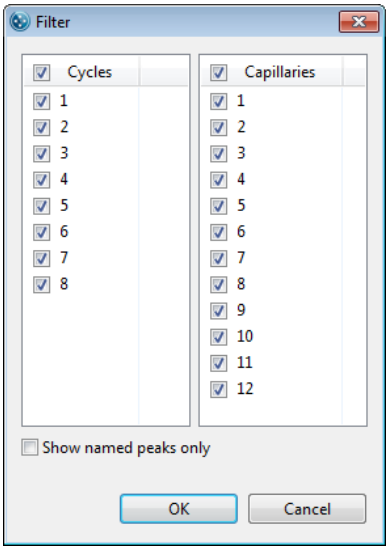
- **To unhide rows** - Select the hidden row(s). Right click on one of the selected rows and click **Unhide.**

Setting Run Data Display Filters

The filter lets you auto-hide specific capillaries in the run file data, or only show data for peaks that Compass identified automatically using the user-defined peak name analysis parameters.

NOTE: When more than one run file is open, filter settings will be applied to all files.

- **To filter data to show specific capillaries only** - Select **View** in the main menu and click **Filter**. Uncheck the boxes for the capillaries you do not want shown, then click **OK**.



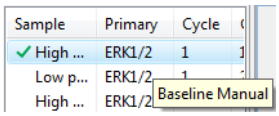
Only data for the checked capillaries will display in data views and results tables, unchecked capillaries will be hidden.

- **To filter data to show named peaks only** - Select **View** in the main menu and click **Filter**. Select **Show named peaks only** then click **OK**. Only data for peaks that Compass identified automatically using the user-defined peak name analysis parameters will display in data views and results tables, all other peak data will be hidden.

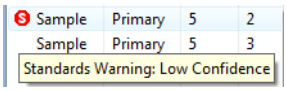
Compass Run Data Notifications and Warnings

If Compass detects a potential data issue, a notification or warning will display next to the row in the experiment pane. A list of warnings, notifications and corrective actions are as follows:

- **Manual correction of sample data notification** - Indicates the user modified sample data manually, such as adding or removing a sample peak. Rolling the mouse over the icon displays the type of data modification made.

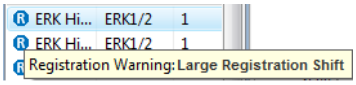


- **Standards warning -** Indicates that one of the standards may not be identified properly. This can be resolved by manually identifying the standards. Please refer to "Step 2 – Checking Fluorescent Sizing Standards" on page 296 for details. Rolling the mouse over the icon displays warning details.



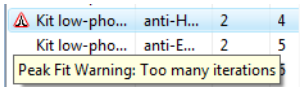
- **Manual correction of standards data notification** - Indicates the user modified the standards data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Registrations warning -** Indicates that a capillary registration was not identified properly. This can be resolved by manually identifying the registration peak. Please refer to "Step 3 – Checking Capillary Registrations" on page 299 for details. Rolling the mouse over the icon displays warning details.



- **Manual correction of registrations notification -** Indicates the user modified the capillary registration data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Peak fit warning -** Indicates that a peak cannot be fit properly. This is typically caused either by a very small peak in the electropherogram or a peak that is very close to the end of the pI range. This can be resolved by removing the peak. Please refer to "Step 4 – Checking Samples" on page 301 or "Step 4 – Checking Samples" on page 301 for details. Rolling the mouse over the icon displays warning details.



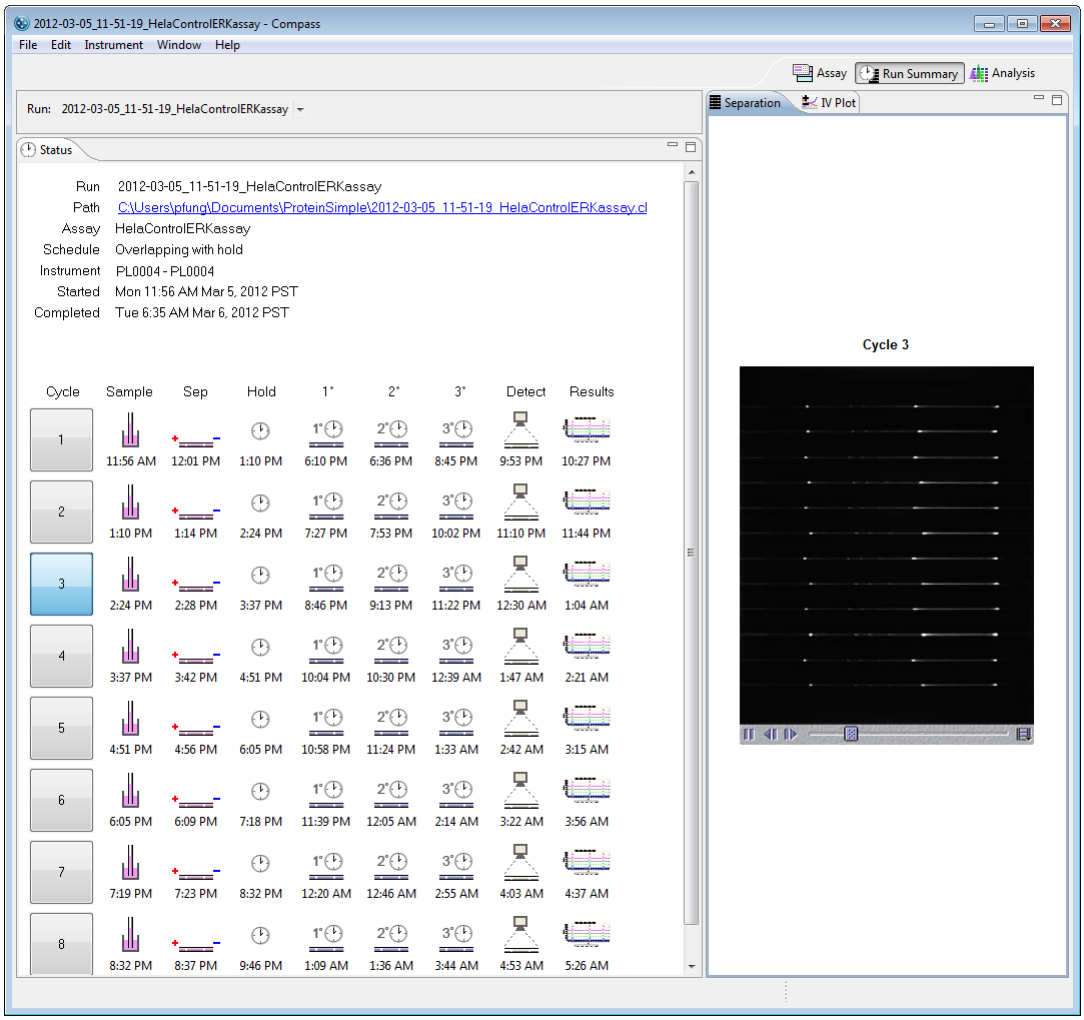
Checking Your Results

Compass detects immunodetected proteins, fluorescent standards and capillary registrations and reports results automatically with no user-interaction. However, ProteinSimple recommends users review their data as outlined in this section as good general practice to ensure accurate results. If a data warning is presented in the experiment pane, the following steps will also help you identify and correct any issues.

Step 1 – Review the Fluorescent Sizing Standards Movie

All capillaries should have three fluorescent sizing standards. To verify the standards separated in the capillary correctly:

1. When the run has completed, click the **Run Summary** screen tab.
2. Click the **Separation** tab and play the movie (this will be the fluorescent standards separation).


2012-03-05_11-51-19_HelaControlERKassay - Compass
File Edit Instrument Window Help
Assay Run Summary Analysis
Run: 2012-03-05_11-51-19_HelaControlERKassay
Status
Run 2012-03-05_11-51-19_HelaControlERKassay
Path C:\Users\pfung\Documents\ProteinSimple\2012-03-05_11-51-19_HelaControlERKassay.cl
Assay HelaControlERKassay
Schedule Overlapping with hold
Instrument PL0004 - PL0004
Started Mon 11:56 AM Mar 5, 2012 PST
Completed Tue 6:35 AM Mar 6, 2012 PST
Cycle Sample Sep Hold 1° 2° 3° Detect Results
1 11:56 AM 12:01 PM 1:10 PM 6:10 PM 6:36 PM 8:45 PM 9:53 PM 10:27 PM
2 1:10 PM 1:14 PM 2:24 PM 7:27 PM 7:53 PM 10:02 PM 11:10 PM 11:44 PM
3 2:24 PM 2:28 PM 3:37 PM 8:46 PM 9:13 PM 11:22 PM 12:30 AM 1:04 AM
4 3:37 PM 3:42 PM 4:51 PM 10:04 PM 10:30 PM 12:39 AM 1:47 AM 2:21 AM
5 4:51 PM 4:56 PM 6:05 PM 10:58 PM 11:24 PM 1:33 AM 2:42 AM 3:15 AM
6 6:05 PM 6:09 PM 7:18 PM 11:39 PM 12:05 AM 2:14 AM 3:22 AM 3:56 AM
7 7:19 PM 7:23 PM 8:32 PM 12:20 AM 12:46 AM 2:55 AM 4:03 AM 4:37 AM
8 8:32 PM 8:37 PM 9:46 PM 1:09 AM 1:36 AM 3:44 AM 4:53 AM 5:26 AM
Separation IV Plot
Cycle 3

3. For each capillary, verify that the standards visibly separated. Each fluorescent point in the capillary represents one of the three sizing standards.

Step 2 – Checking Fluorescent Sizing Standards

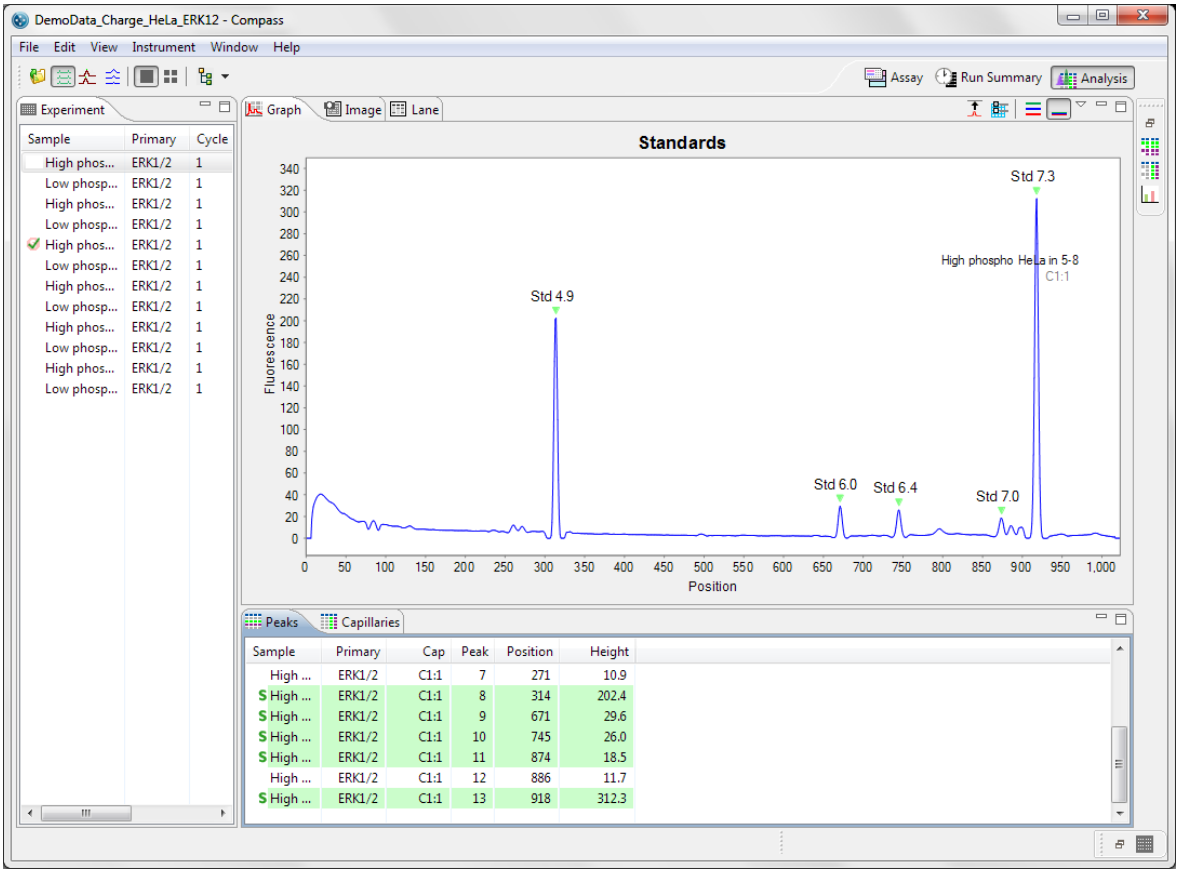
To verify the standards are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Standards** in the View bar. Verification that the standards have been correctly identified can be done in either the graph or lane panes.

Graph Pane:

a. Click **Single View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Graph** tab. Check that the electropherogram has the appropriate number of fluorescent pI standard peaks for the pI Standard Ladder you are using. They will also be identified with a green **S** in the peaks table. The pI standards at the low and high end of the pI range in the electropherogram are at higher concentrations as they are also used for capillary registration. In the following example, the pI standards shown are those for pI Standard Ladder 3 (P/N 040-646).



Sample	Primary	Cycle
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1

Sample	Primary	Cap	Peak	Position	Height
High ...	ERK1/2	C1:1	7	271	10.9
S High ...	ERK1/2	C1:1	8	314	202.4
S High ...	ERK1/2	C1:1	9	671	29.6
S High ...	ERK1/2	C1:1	10	745	26.0
S High ...	ERK1/2	C1:1	11	874	18.5
High ...	ERK1/2	C1:1	12	886	11.7
S High ...	ERK1/2	C1:1	13	918	312.3

If standards are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a standard** - Right click the peak in the electropherogram or peaks table and select **Not a Standard**. Compass should correctly reassign the remaining peaks as standards, and update the peaks table.

- **To set an unidentified peak as a standard** - Right click the peak in the electropherogram or peaks table and select **Force Standard**. Compass will assign the peak as a standard, and correctly reassign the remaining standard peaks. A lock icon indicating the standard was set manually will display next to the peak in the peaks table.

---

*NOTE: To remove standards peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Clear**.*

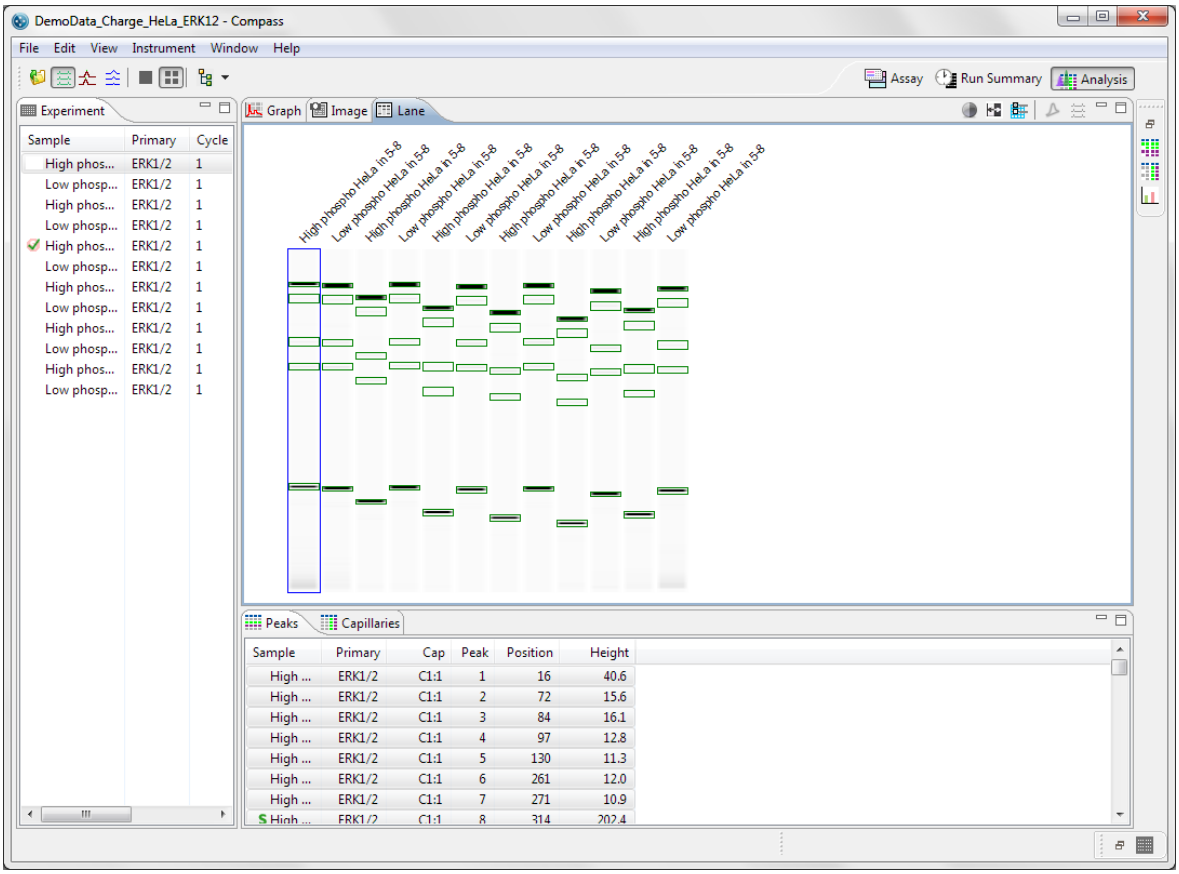
---

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Lane Pane:

a. Click **Multiple View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Lane** tab. Standards will be bands and identified with a green outline. Check that each lane has the appropriate number of fluorescent pI standard bands for the pI Standard Ladder you are using. They will also be identified with a green **S** in the peaks table. The pI standard bands at the low and high end of the pI range in each lane will display greater intensity as they are at higher concentrations and are also used as registration standards. In the following example, the pI standards shown are those for pI Standard Ladder 3 (P/N 040-646). To view band labels, roll the mouse over the individual bands.



If standards are not identified correctly, they can be manually corrected as follows:

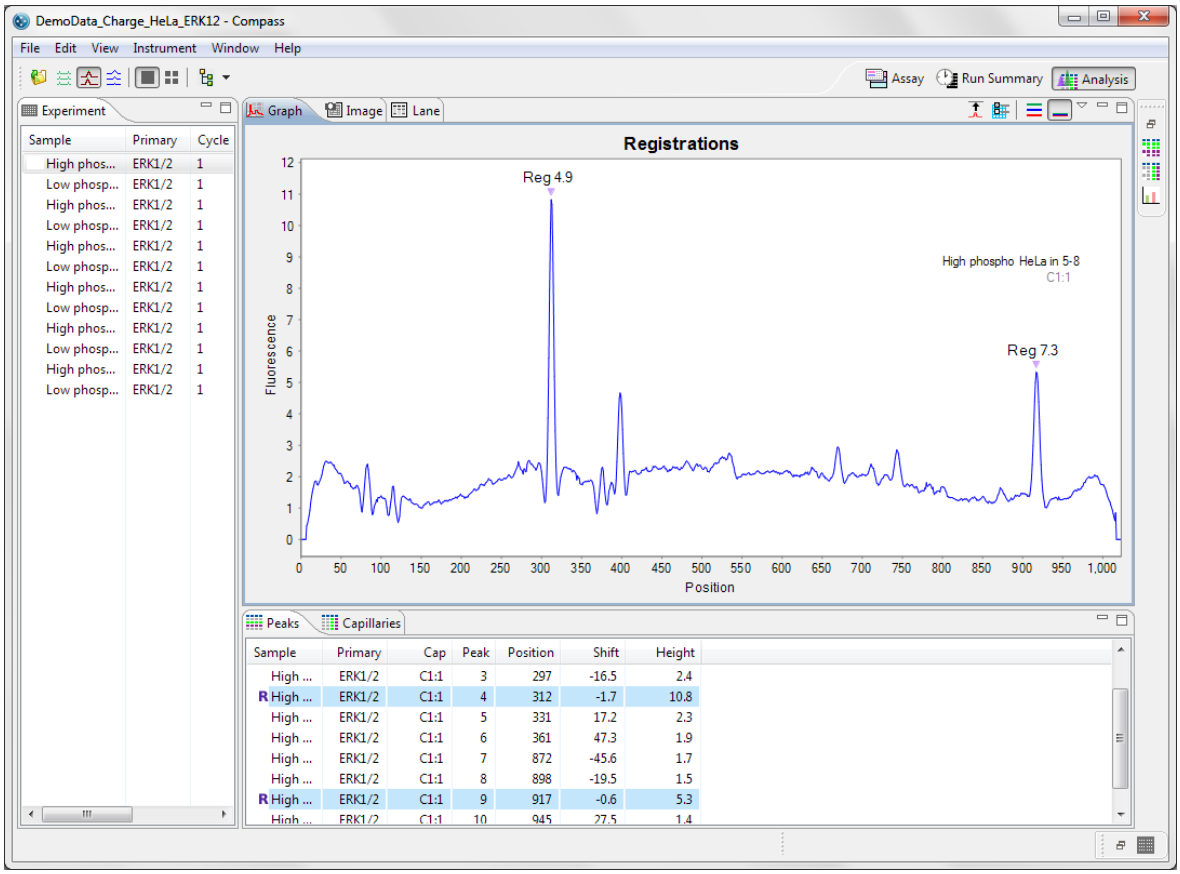
- **If an incorrect band is identified as a standard** - Right click the band in the lane or peaks table and select **Not a Standard**. Compass should correctly reassign the remaining bands as standards.
- **To set an unidentified band as a standard** - Right click the band in the lane or peaks table and select **Force Standard**. Compass will assign the band as a standard, and correctly reassign the remaining standard bands.

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Step 3 – Checking Capillary Registrations

All capillaries should have a single capillary registration peak. To verify the registrations are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Registrations** and **Single View** in the View bar.
3. Click the **Graph** tab.
4. Click on the first row in the experiment pane. The registration peak will be the first and largest peak in the electropherogram. Check that the two registration peaks are identified and labeled Reg 1 and Reg 2 in the electropherogram. They will also be identified with a purple **R** in the peaks table.



5. If the registration peak is not identified correctly, right click the correct registration peak in the electropherogram or peaks table and select **Registration Force**. Compass will assign the new peak as the capillary registration. A lock icon indicating the registration was set manually will display next to the peak in the peaks table.

*NOTE: To remove registration peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Registration Clear**.*

6. Repeat the previous steps for the remaining rows in the experiment pane to make sure all registrations are identified correctly.

Step 4 – Checking Samples

All immunodetected sample proteins in the graph and lane panes will be labeled automatically with the calculated protein pI.

NOTE: The reported pI for immunodetected sample proteins in Compass may vary slightly from predicted pIs based on sample, buffer and assay conditions.

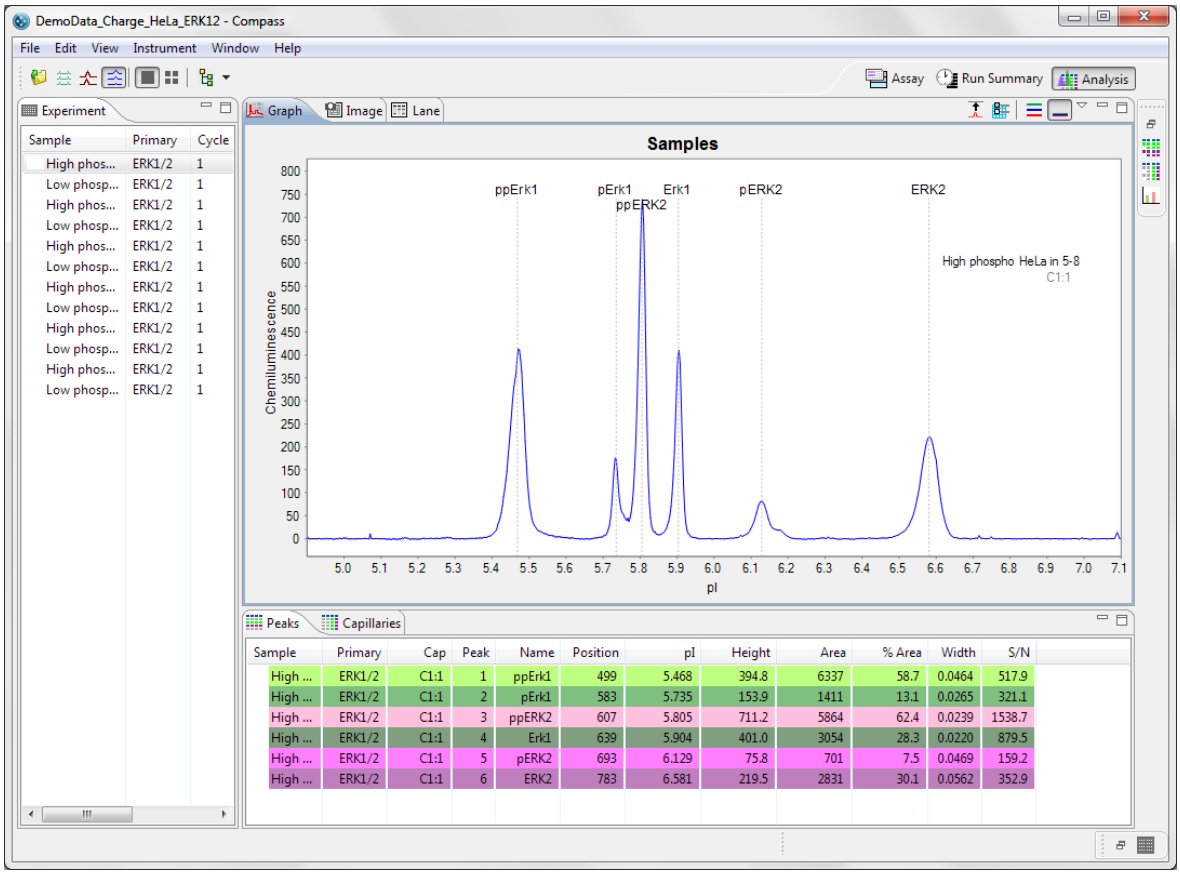
To verify that sample proteins are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Samples** in the View bar. Verification that sample proteins have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

   Graph Pane:

   a. Click **Single View** in the View bar.

   b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Graph** tab.



Sample	Primary	Cycle
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1
High phos...	ERK1/2	1
Low phosp...	ERK1/2	1

Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
High ...	ERK1/2	C1:1	1	ppErk1	499	5.468	394.8	6337	58.7	0.0464	517.9
High ...	ERK1/2	C1:1	2	pErk1	583	5.735	153.9	1411	13.1	0.0265	321.1
High ...	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7
High ...	ERK1/2	C1:1	4	Erk1	639	5.904	401.0	3054	28.3	0.0220	879.5
High ...	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2
High ...	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9

If sample peaks are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.
- **To set an unidentified peak as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

---

*NOTE: To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

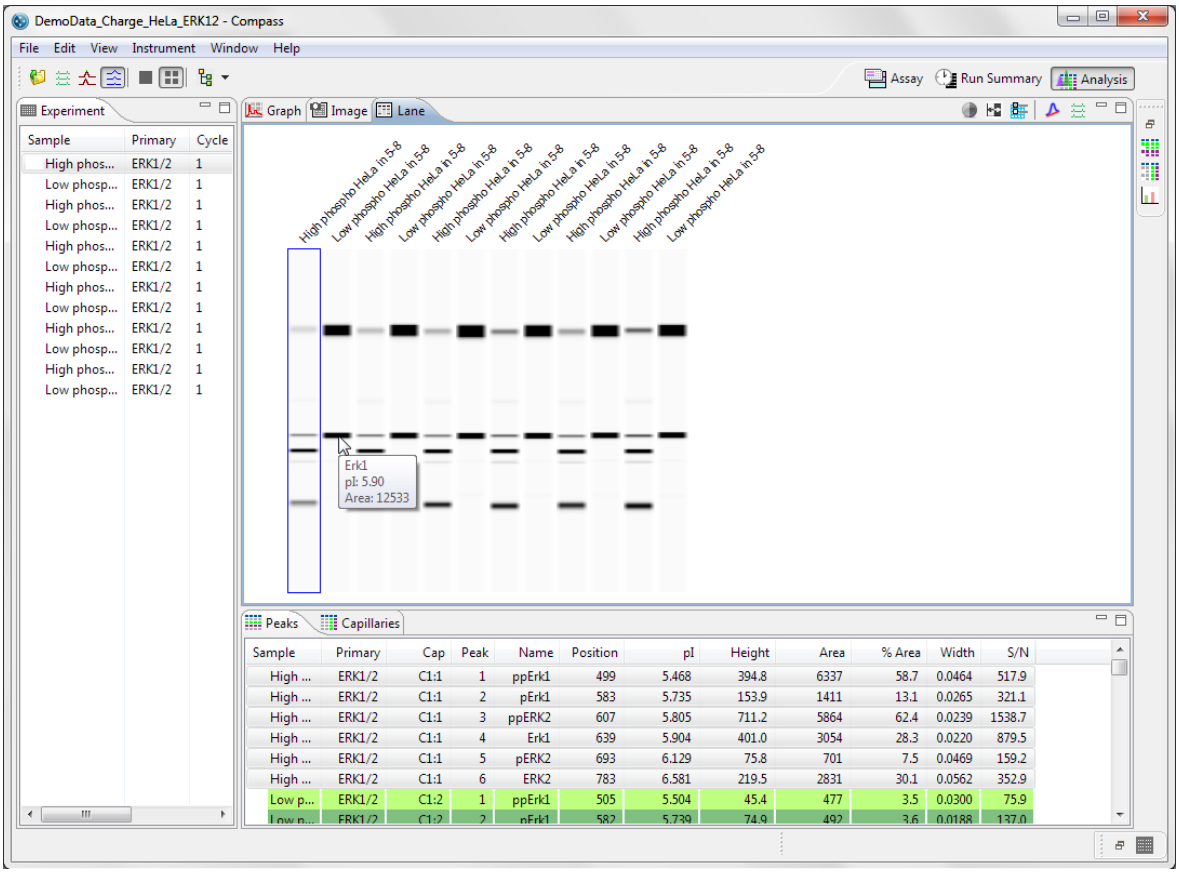
---

c. Repeat the previous steps for the remaining sample rows in the experiment pane to make sure all sample proteins are identified correctly.

Lane Pane:

a. Click either **Single View** or **Multiple View** in the View bar.

b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Lane** tab. To view sample protein band labels, roll the cursor over the individual bands. If sample bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.


DemoData_Charge_HeLa_ERK12 - Compass
File Edit View Instrument Window Help
Assay Run Summary Analysis
Experiment
Graph Image Lane
Sample Primary Cycle
Erk1
pI: 5.90
Area: 12533
Peaks Capillaries
Sample Primary Cap Peak Name Position pI Height Area % Area Width S/N
High ... ERK1/2 C1:1 1 ppErk1 499 5.468 394.8 6337 58.7 0.0464 517.9
High ... ERK1/2 C1:1 2 pErk1 583 5.735 153.9 1411 13.1 0.0265 321.1
High ... ERK1/2 C1:1 3 ppERK2 607 5.805 711.2 5864 62.4 0.0239 1538.7
High ... ERK1/2 C1:1 4 Erk1 639 5.904 401.0 3054 28.3 0.0220 879.5
High ... ERK1/2 C1:1 5 pERK2 693 6.129 75.8 701 7.5 0.0469 159.2
High ... ERK1/2 C1:1 6 ERK2 783 6.581 219.5 2831 30.1 0.0562 352.9
Low p... ERK1/2 C1:2 1 ppErk1 505 5.504 45.4 477 3.5 0.0300 75.9

Step 5 – Assigning Peak Names (Optional)

Compass can identify and automatically name sample proteins associated with specific primary antibodies in the run data using user-specified peak name settings. For more information on how to do this see "Peak Names Settings" on page 367.

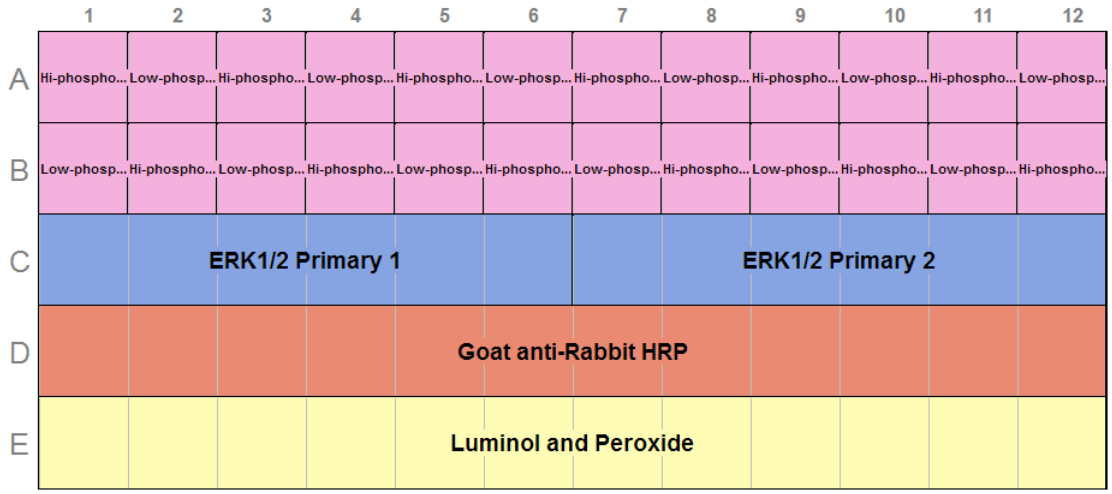
Group Statistics

The Grouping View is used to analyze replicates by calculating the mean, standard deviation, %CV and SEM for named proteins (see "Peak Names Settings" on page 367 for detailed information on entering named proteins). Statistics for each protein are also plotted for easy comparison between samples, antibodies, and proteins.

Using Groups

1. A group is automatically created for capillaries with the same sample and primary antibody name, so to use this feature, this information must first be entered into the Assay template. To do this:
   a. Click the **Assay** tab and go to the Template pane.
   b. Enter sample names and primary antibody names as described in "Step 6 - Add Assay Plate Annotations (Optional)" on page 79. Be sure to enter the same sample and/or primary antibody names for the groups of samples you want to calculate statistics for.

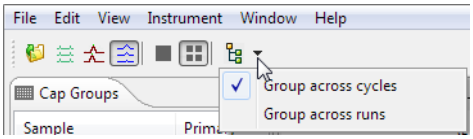
      In this example there are two sample types, **High phospho HeLa** and **Low phospho HeLa** which were run with two different antibodies, **ERK 1/2 Primary 1** and **ERK 1/2 Primary 2**.



      Each of the two samples were run with the two antibodies in every cycle, and the ERK1/2 antibody generates 6 named peaks.

2. Select the **Analysis** tab and click the **Grouping View** icon in the view bar. Clicking this icon toggles the grouping view on and off.

3. Select a grouping option by clicking the arrow next to the grouping view icon. These options allow you to group capillaries in multiple ways:

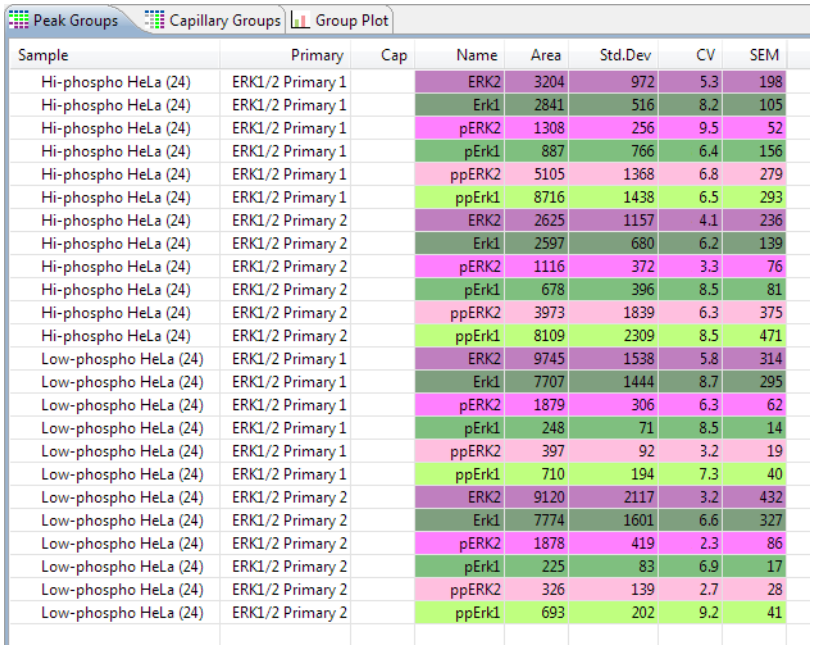


- **Group across cycles** - Groups capillaries run in different cycles.
- **Group across runs** - Groups capillaries from multiple runs. This option creates fewer groups and generates statistics across multiple runs.
- **No option selected** - When only one run is open, groups will only contain capillaries from the same cycle. When more than one run is open, groups are created for each run.

Viewing Statistics

Peak and Capillary Groups

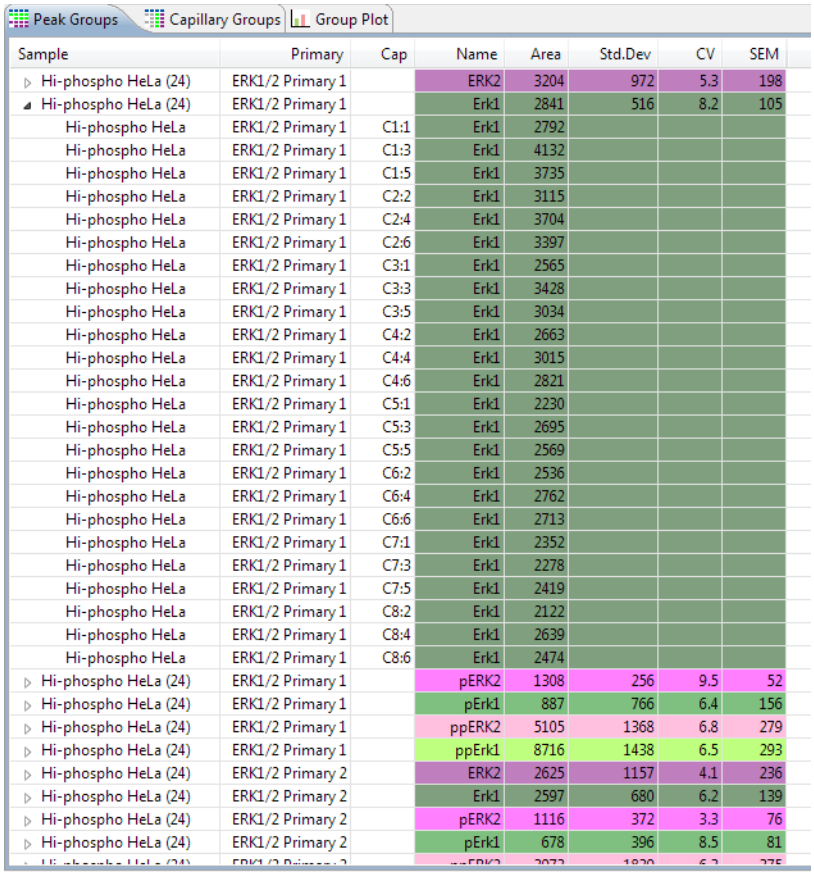
The Peak Groups pane reports statistics for each named protein in every group. Each group shows the statistics for named proteins which includes average area, standard deviation, %CV and SEM. The number in parenthesis after the sample name indicates the number of capillaries in the group.



Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Cap	Name	Area	Std.Dev	CV	SEM
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	3204	972	5.3	198
Hi-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	2841	516	8.2	105
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1308	256	9.5	52
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	887	766	6.4	156
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	5105	1368	6.8	279
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	8716	1438	6.5	293
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	2625	1157	4.1	236
Hi-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	2597	680	6.2	139
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1116	372	3.3	76
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	678	396	8.5	81
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ppERK2	3973	1839	6.3	375
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ppErk1	8109	2309	8.5	471
Low-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	9745	1538	5.8	314
Low-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	7707	1444	8.7	295
Low-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1879	306	6.3	62
Low-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	248	71	8.5	14
Low-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	397	92	3.2	19
Low-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	710	194	7.3	40
Low-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	9120	2117	3.2	432
Low-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	7774	1601	6.6	327
Low-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1878	419	2.3	86
Low-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	225	83	6.9	17
Low-phospho HeLa (24)	ERK1/2 Primary 2		ppERK2	326	139	2.7	28
Low-phospho HeLa (24)	ERK1/2 Primary 2		ppErk1	693	202	9.2	41

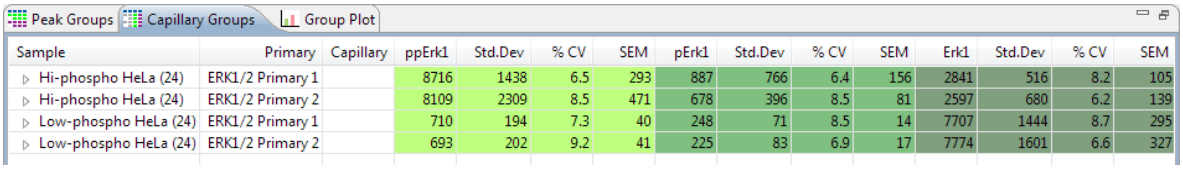
Clicking the arrow next to a group lists the individual capillaries in the group and reported data for each capillary:



Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Cap	Name	Area	Std.Dev	CV	SEM
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	3204	972	5.3	198
Hi-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	2841	516	8.2	105
Hi-phospho HeLa	ERK1/2 Primary 1	C1:1	Erk1	2792			
Hi-phospho HeLa	ERK1/2 Primary 1	C1:3	Erk1	4132			
Hi-phospho HeLa	ERK1/2 Primary 1	C1:5	Erk1	3735			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:2	Erk1	3115			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:4	Erk1	3704			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:6	Erk1	3397			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:1	Erk1	2565			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:3	Erk1	3428			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:5	Erk1	3034			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:2	Erk1	2663			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:4	Erk1	3015			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:6	Erk1	2821			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:1	Erk1	2230			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:3	Erk1	2695			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:5	Erk1	2569			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:2	Erk1	2536			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:4	Erk1	2762			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:6	Erk1	2713			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:1	Erk1	2352			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:3	Erk1	2278			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:5	Erk1	2419			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:2	Erk1	2122			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:4	Erk1	2639			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:6	Erk1	2474			
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1308	256	9.5	52
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	887	766	6.4	156
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	5105	1368	6.8	279
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	8716	1438	6.5	293
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	2625	1157	4.1	236
Hi-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	2597	680	6.2	139
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1116	372	3.3	76
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	678	396	8.5	81

The **Capillary Groups** pane pivots the **Peak Groups** results to show statistics for named protein peaks in individual columns.

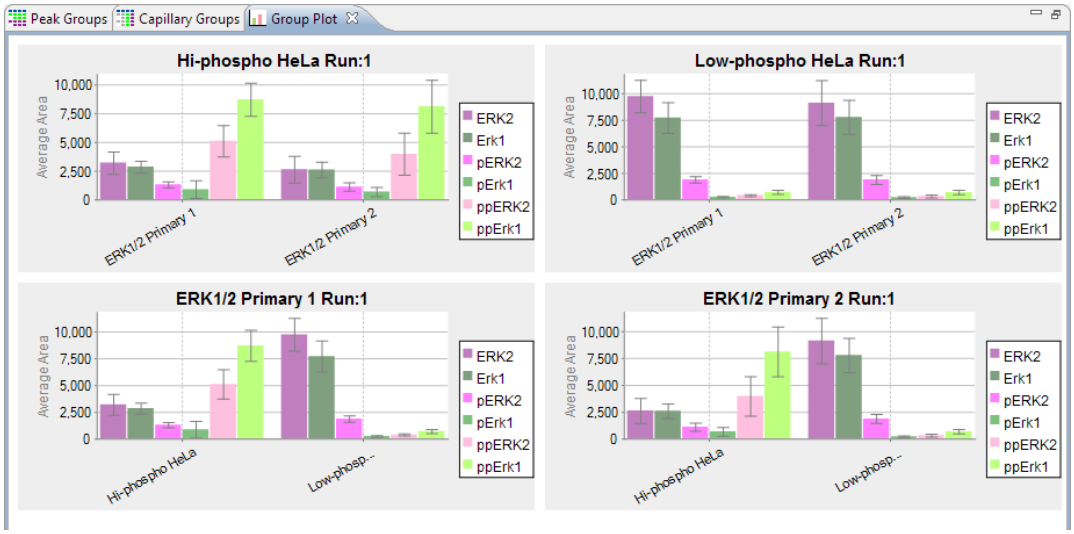


Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Capillary	ppErk1	Std.Dev	% CV	SEM	pErk1	Std.Dev	% CV	SEM	Erk1	Std.Dev	% CV	SEM
Hi-phospho HeLa (24)	ERK1/2 Primary 1		8716	1438	6.5	293	887	766	6.4	156	2841	516	8.2	105
Hi-phospho HeLa (24)	ERK1/2 Primary 2		8109	2309	8.5	471	678	396	8.5	81	2597	680	6.2	139
Low-phospho HeLa (24)	ERK1/2 Primary 1		710	194	7.3	40	248	71	8.5	14	7707	1444	8.7	295
Low-phospho HeLa (24)	ERK1/2 Primary 2		693	202	9.2	41	225	83	6.9	17	7774	1601	6.6	327

Group Plots

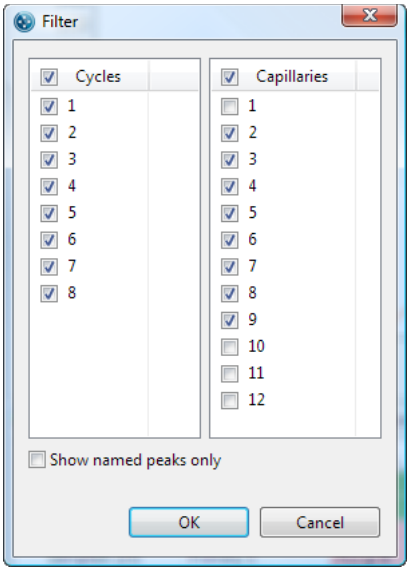
The mean values for named peaks in each group are plotted in bar graphs with error bars showing the standard deviation. The plots compare different antibodies for the same sample and different samples for the same antibody to allow a choice of presentation.



Hiding or Removing Capillaries in Group Analysis

Hidden capillaries are not included in groups. However, hiding capillaries provides an easy way to reject individual capillaries from the statistical analysis. See “Hiding Capillary Data” on page 291 for details on how to do this.

Larger groups of capillaries can also be excluded from analysis. To do this, select **View** and click **Filter**.



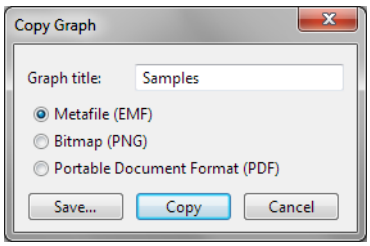
Uncheck the box next to the cycles or capillaries you wish to remove and click **OK**. This data will now be removed from the grouped view statistics.

Copying Data Views and Results Tables

You can copy and paste data and results tables into other documents, or save a data view as a graphic file.

Copying Data Views

1. Click in the graph or lane pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. If you selected copy from the graph pane the following window will display, click **Copy**. If you selected copy from the lane pane skip to the next step.



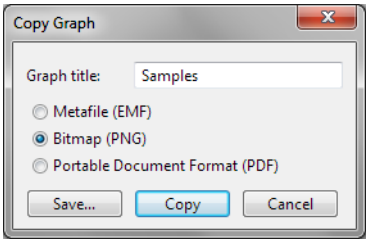
4. Open a document (Word, Excel, PowerPoint, etc). Right click in the document and select **Paste**. A graphic of the copied data view will be pasted into the document.

Copying Results Tables

1. Click in the peaks or capillaries pane.
2. Select one or multiple rows.
3. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
4. Open a document (Word, Excel, PowerPoint, etc). Right click in the document and select **Paste**. Data for the rows selected will be pasted into the document.

Saving the Graph View as an Image File

1. Click in the graph pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. Select an image option (EMF, PNG or PDF) in the pop-up window, then click **Save**.



4. Select a directory to save the file to and enter a file name, then click **OK**.

Exporting Run Files

Results tables and raw plot data can be exported for use in other applications.

Exporting Results Tables

To export the information in the peaks and capillaries tables:

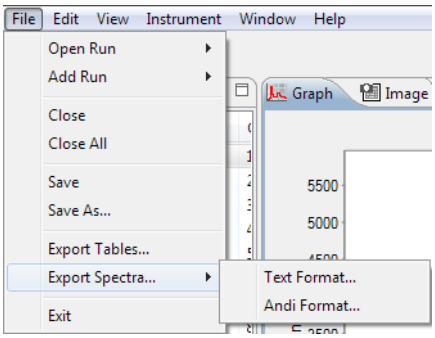
1. Click **File** in the main menu and click **Export Tables**.
2. Select a directory to save the files to and click **OK**. Data will be exported in .txt format.

NOTE: To exclude export of standards data or export results table data in .csv format, see "Setting Data Export Options" on page 391.

Exporting Raw Sample Electropherogram Data

To export raw sample plot data:

1. Click **File** in the main menu and click **Export Spectra**.



- **To export data in .txt format** - Select **Text Format**. Plots will be exported in one file for all capillaries.
- **To export data in .cdf format** - Select **Andi Format**. Plots will be exported in one file per capillary.

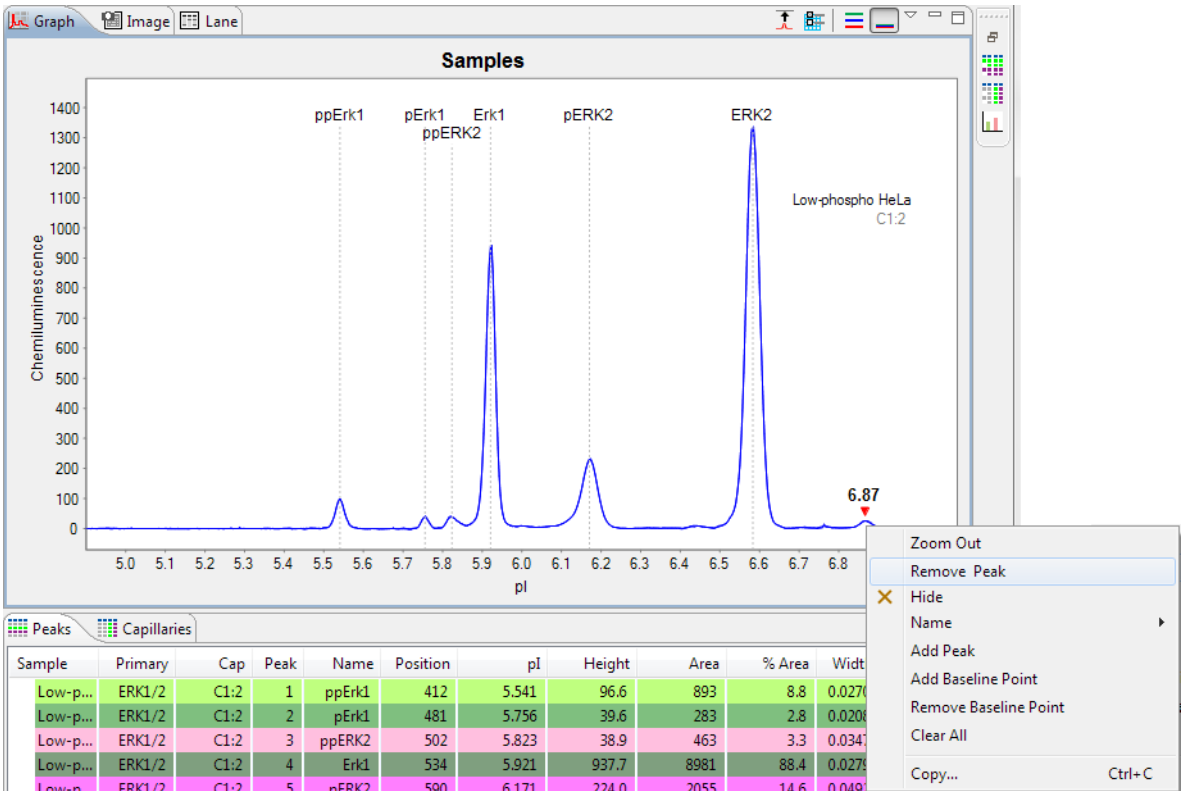
2. Select a directory to save the files to and click **OK**. Data will be exported in the selected format.

Changing Sample Protein Identification

Compass allows you to customize what sample proteins are reported in the results tables by making manual adjustments in the electropherogram or peaks table.

Adding or Removing Sample Data

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
    - **To remove a peak from the data -** Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.



- **To add an unidentified peak to the data -** Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

NOTES:

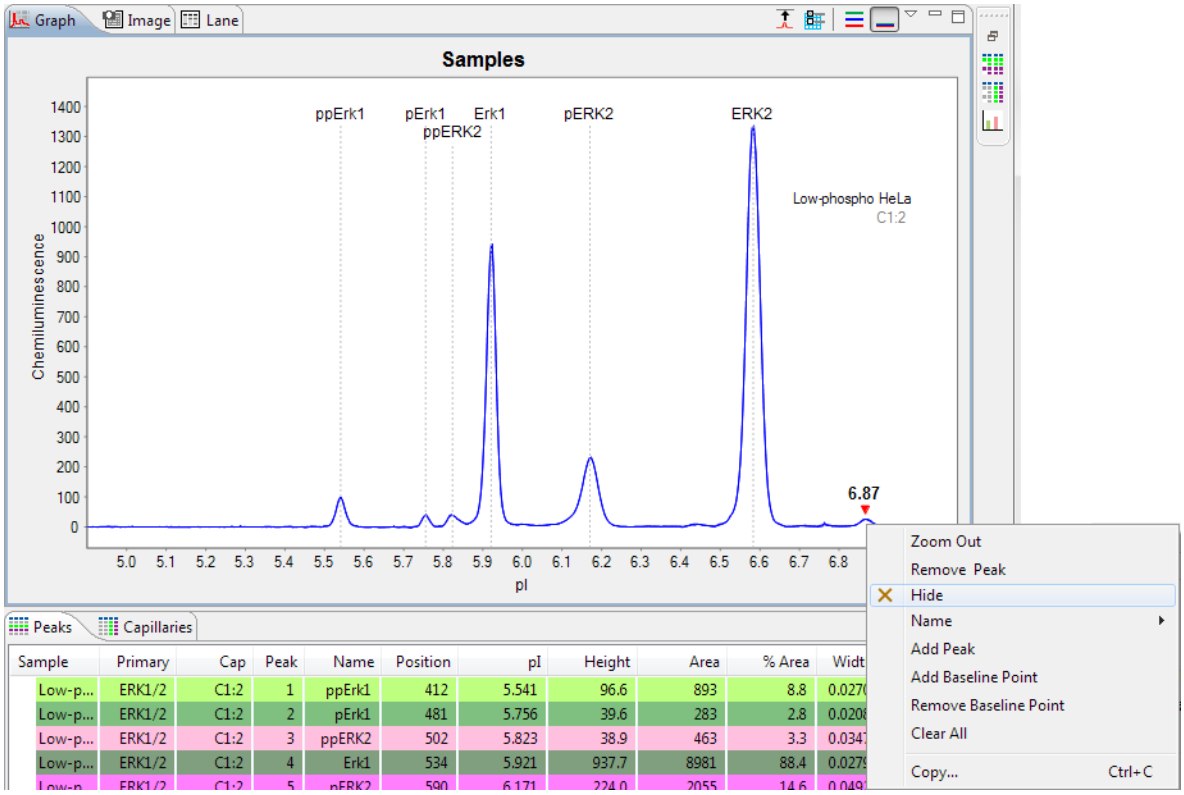
To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click ***Clear All****.*

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

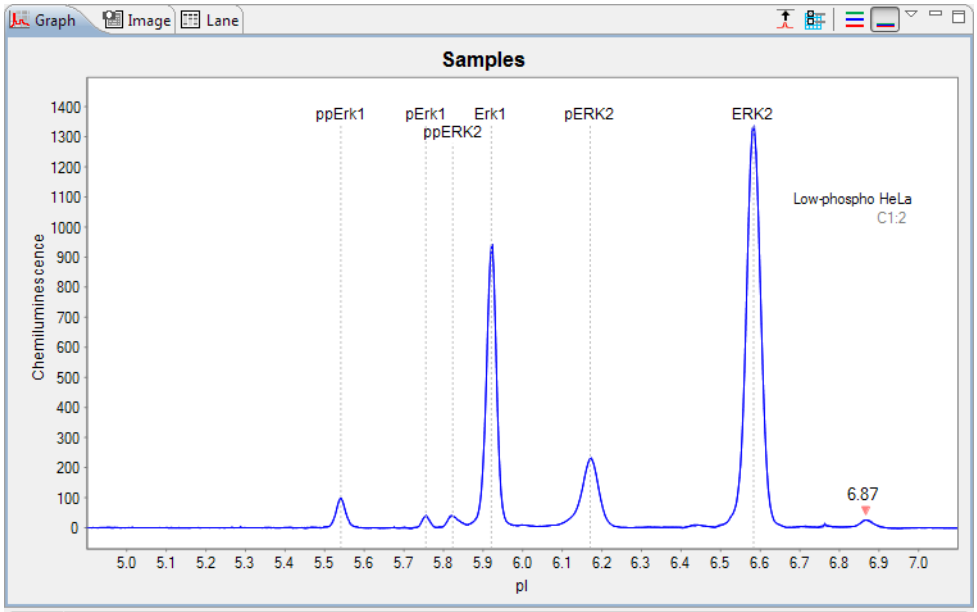
Hiding Sample Data

You can hide the results for a sample protein in the results tables without completely removing it from the reported results. To to this:

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
4. Right click the peak in the electropherogram or peaks table and select **Hide peak**. Compass will hide the peak data in the results tables.



5. To view hidden peak data, click **View** in the main menu and click **Show Hidden**. Hidden peak data will display in the results table and be marked with an **X**.



Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Low-p...	ERK1/2	C1:2	1	ppErk1	412	5.541	96.6	893	8.8	0.0270	231.2
Low-p...	ERK1/2	C1:2	2	pErk1	481	5.756	39.6	283	2.8	0.0208	103.8
Low-p...	ERK1/2	C1:2	3	ppERK2	502	5.823	38.9	463	3.3	0.0347	79.1
Low-p...	ERK1/2	C1:2	4	Erk1	534	5.921	937.7	8981	88.4	0.0279	2218.7
Low-p...	ERK1/2	C1:2	5	pERK2	590	6.171	224.0	2055	14.6	0.0492	542.4
Low-p...	ERK1/2	C1:2	6	ERK2	667	6.583	1345.5	11523	82.1	0.0398	3382.2
Low-p...	ERK1/2	C1:2	7		724	6.869	25.6	212		0.0385	63.5

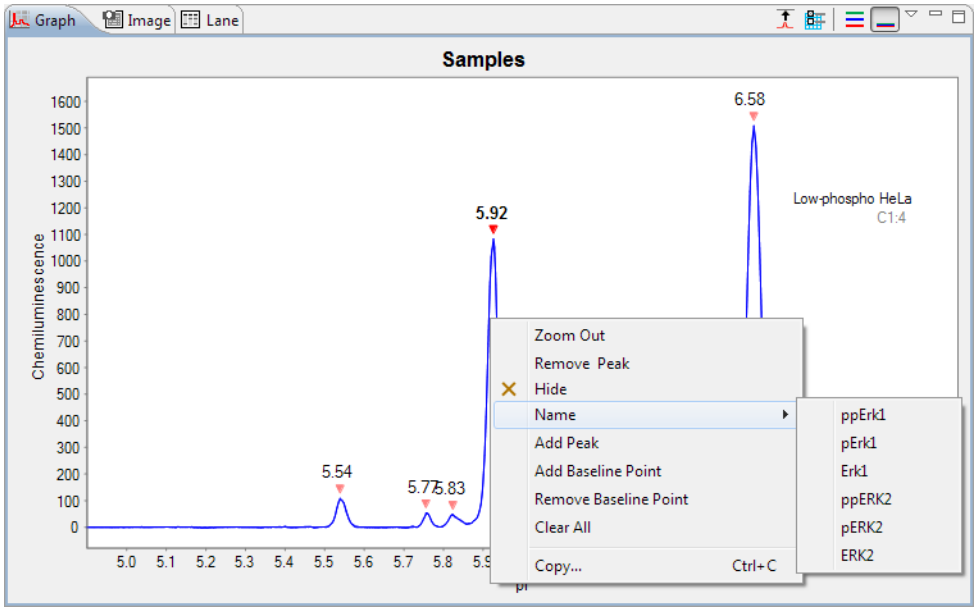
6. To unhide a peak, right click on the peak in the electropherogram or peaks table and select **Unhide Peak**.

Changing Peak Names for Sample Data

If Compass did not automatically name a sample protein associated with a specific primary antibody, this can be adjusted manually. To do this:

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.

4. Right click the peak in the electropherogram or peaks table and click **Name**, then click a name in the list. Compass will change the peak name in the electropherogram and results tables, and adjust peak names for other sample proteins accordingly.



NOTES:

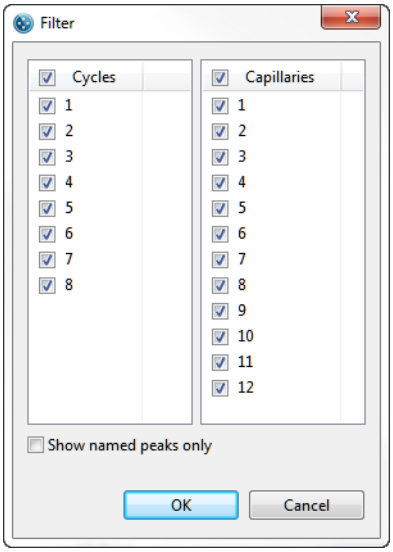
For details on how to specify peak name settings, see "Peak Names Settings" on page 367.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

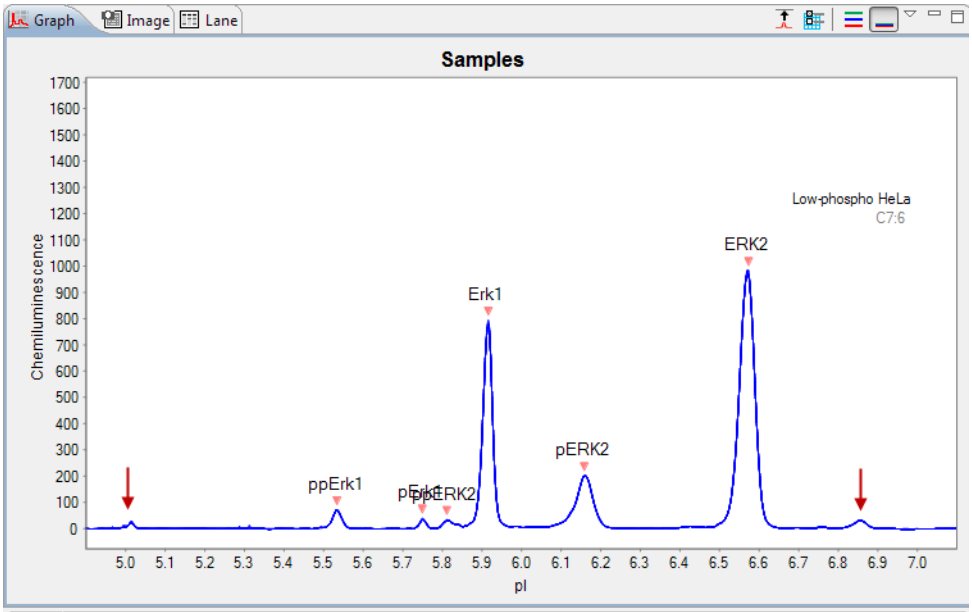
Displaying Sample Data for Named Peaks Only

You can adjust the sample data to display results only for user-specified named peaks. To do this:

1. Click **Show Samples** in the View bar.
2. Click **View** in the main menu and click **Filter**.
3. Check the **Show Named Peaks only** box and click **OK**.



Compass will hide peak data in the electropherogram and results tables for all unnamed peaks, and instead only display data for named peaks in the electropherogram and results tables.



Peaks | Capillaries

Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Low-p...	ERK1/2	C7:6	2	ppErk1	352	5.534	70.3	628	7.7	0.0259	159.9
Low-p...	ERK1/2	C7:6	3	pErk1	422	5.750	38.3	230	2.8	0.0174	89.8
Low-p...	ERK1/2	C7:6	4	ppERK2	442	5.815	30.0	375	3.3	0.0361	59.6
Low-p...	ERK1/2	C7:6	5	Erk1	476	5.914	788.7	7286	89.5	0.0268	1764.7
Low-p...	ERK1/2	C7:6	6	pERK2	531	6.159	194.8	1811	15.8	0.0498	436.5
Low-p...	ERK1/2	C7:6	7	ERK2	608	6.570	984.9	9257	80.9	0.0430	2195.6

Changing the Virtual Blot View

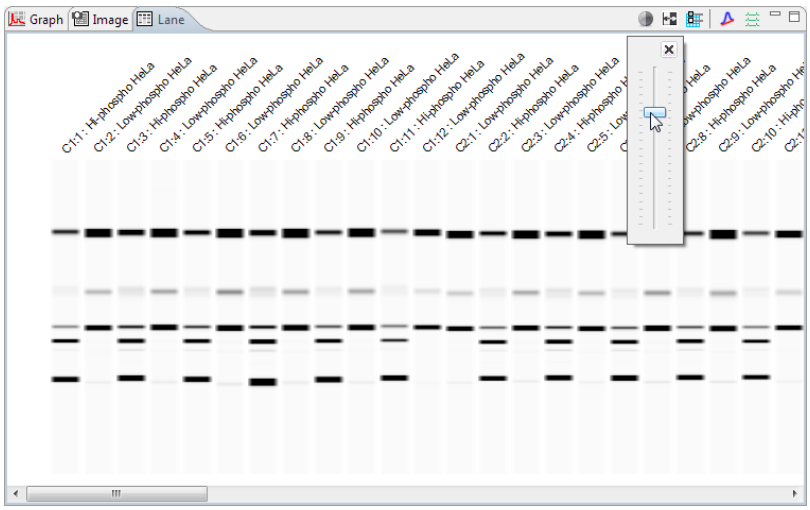
Options in the lane pane let users change the contrast or invert the virtual blot, remove baseline noise, change lane labels or overlay standards data on sample lanes.

The lane pane toolbar has the following options:

- Contrast Adjustment
- Invert
- Edit Labels
- Remove Baseline
- Overlay Standards Data

Adjusting the Contrast

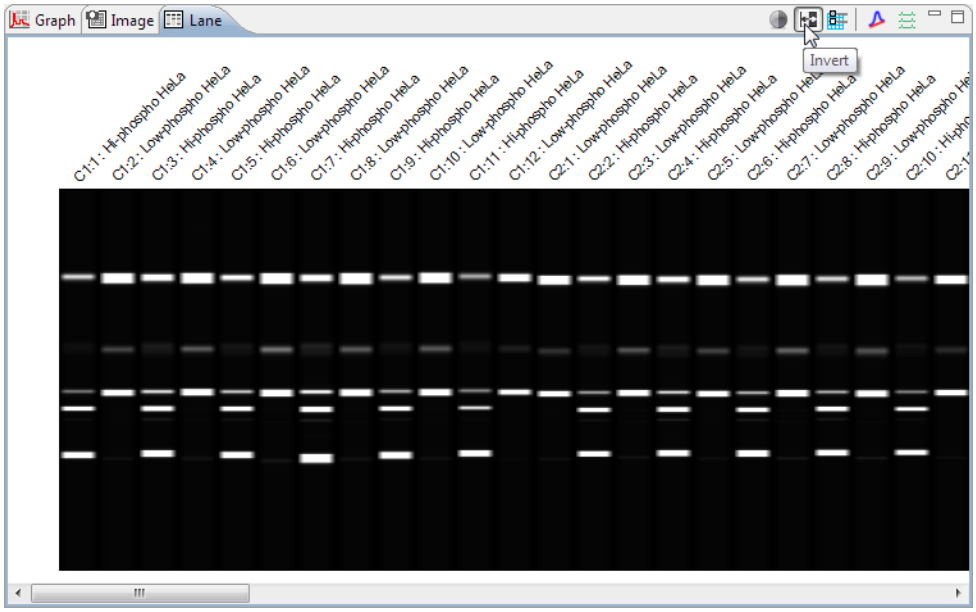
1. Click the **Contrast Adjustment** button. The contrast tool will display:



2. Click the bar and drag it up or down to adjust the contrast.
3. When finished, click **X** to close the tool.

Inverting the Virtual Blot

1. Click the **Invert** button. The virtual blot image will invert:

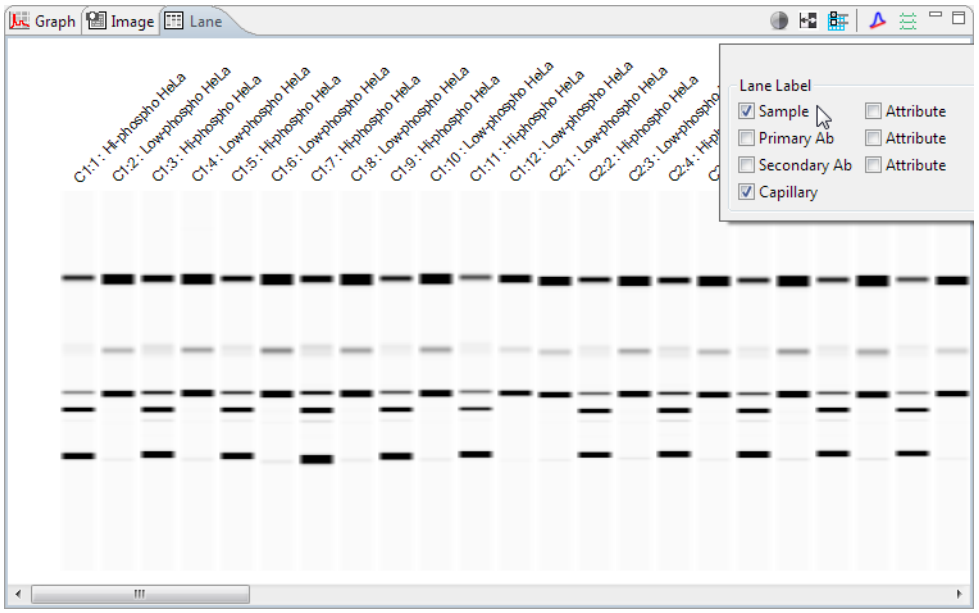


2. Click the **Invert** button again to return to the default view.

Selecting Lane Labels

The labels shown above the lanes in the virtual blot can be customized. To do this:

1. Click the **Edit Labels** button. The label box will display:



2. Check one or multiple label boxes, and uncheck those you don't want to display. To remove labels completely, uncheck all boxes. The following label options are available:

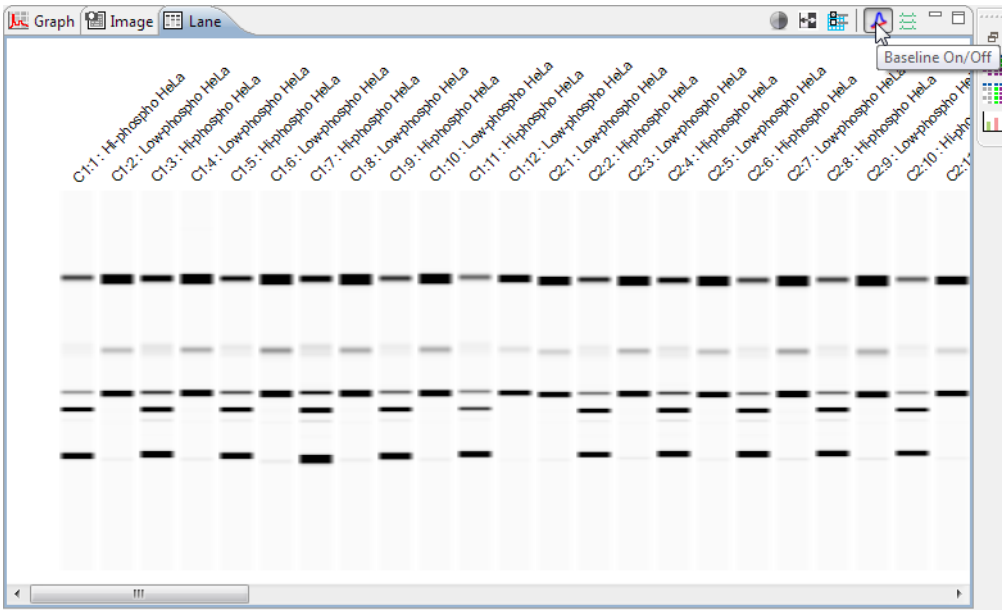
   - **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
   - **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
   - **Secondary Ab** - Secondary antibody name. If a secondary antibody name was entered in the assay template (Assay screen), those names will display here. Otherwise, Secondary (default name) will display.
   - **Capillary** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3

NOTE: Peggy runs up to eight cycles (12 capillaries at a time), so the cycle number displayed will always be C1-C8 depending on the number of cycles programmed.

   - **Attributes** - Attribute text. If attribute information was entered for any of the rows or wells in the assay template (Assay screen), up to four can be selected and displayed as lane labels.

Viewing the Uncorrected Sample Baseline

1. Click the **Remove Baseline** button (active for sample data only). This will remove the automatic baseline correction.

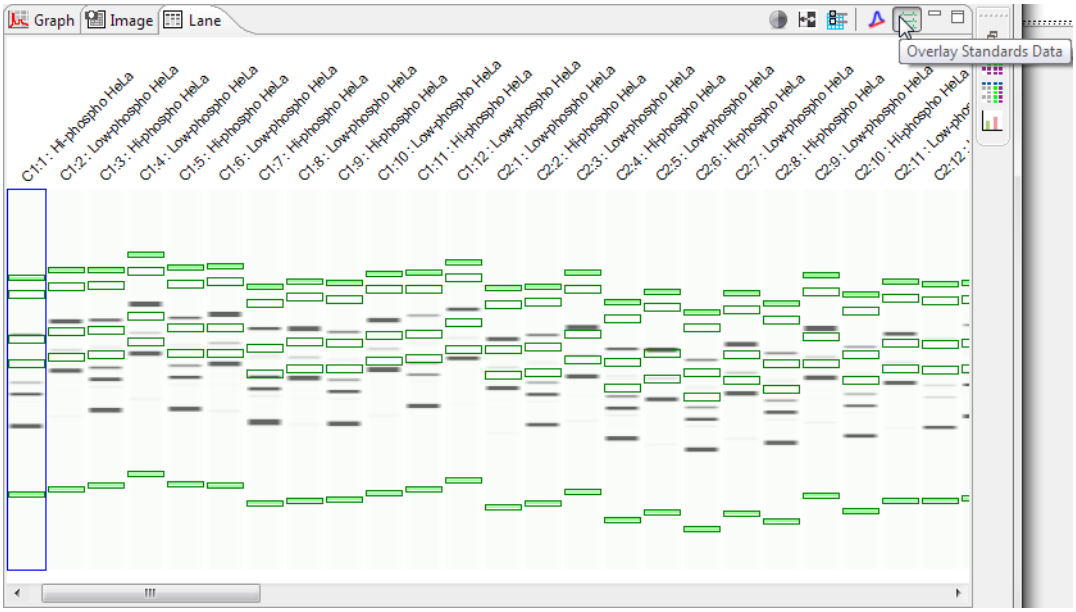


2. Click the **Remove Baseline** button again to return to the default, baseline corrected view.

Overlaying Standards Data on Sample Lanes

Data for the standards can be overlaid on the sample data in the virtual blot so users can view the raw, unaligned (uncorrected) lane data. To do this:

1. Click the **Overlay Standards Data** button (active for sample data only). An overlay of the raw sample and standards data will display:

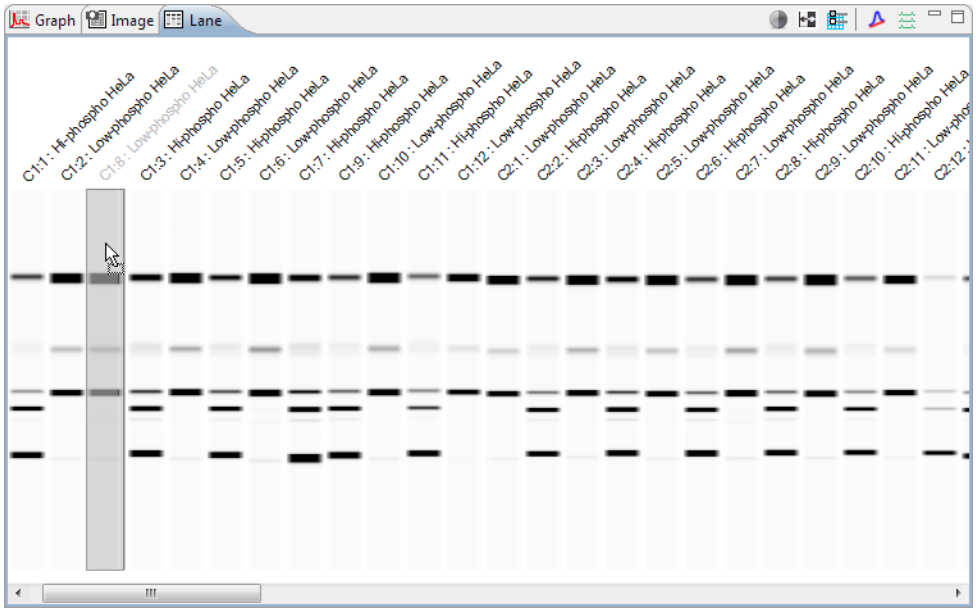


2. Click the **Overlay Standards Data** button again to return to the default, auto-aligned view.

Moving Lanes in the Virtual Blot View

Lanes in the virtual blot view can be moved to make it easier to compare specific lanes of data. To do this:

1. Click a lane in the virtual blot. Hold the mouse button down and drag the lane to a new position.



2. Release the mouse button. The lane will now be repositioned in the virtual blot view.

Changing the Electropherogram View

Options in the graph pane let users zoom and scale electropherograms, overlay or stack plots and change the peak and plot information displayed.

The graph pane toolbar has the following options:

Auto Scale

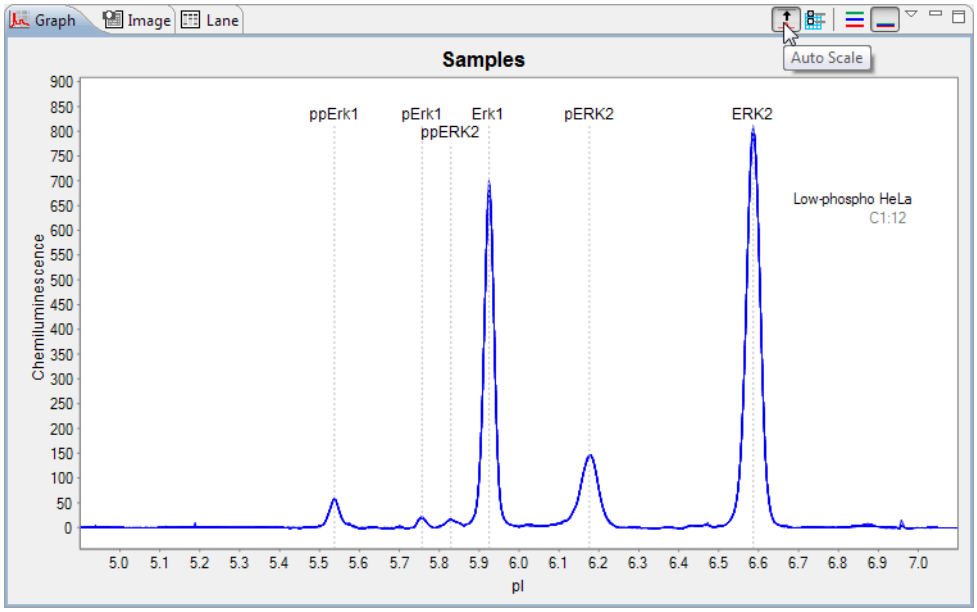
Graph Options

Stack the Plots

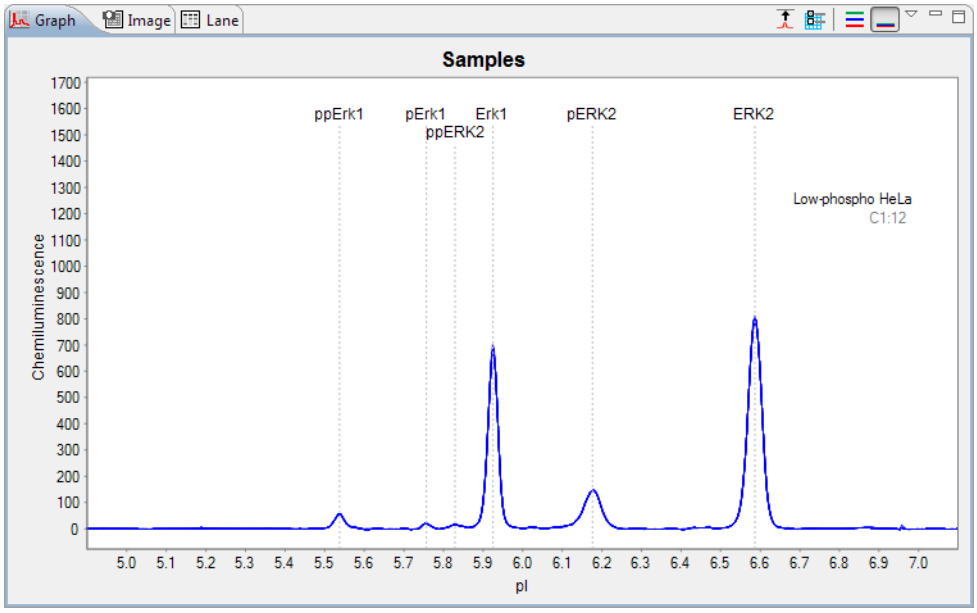
Overlay the Plots

Autoscaling the Electropherogram

Click the **Autoscale** button to scale the y-axis to the largest peak in the electropherogram.



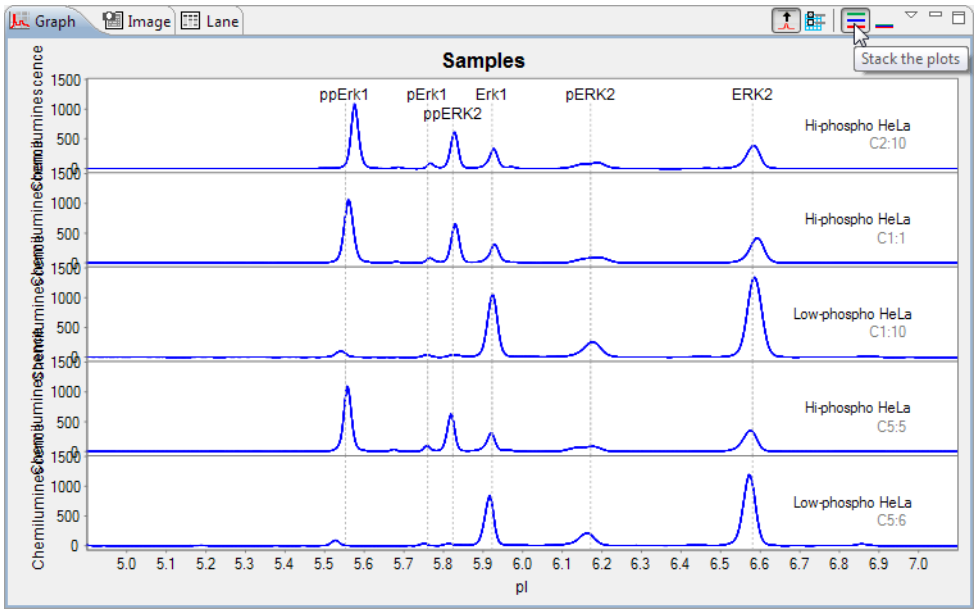
Click the **Autoscale** button again to return to default scaling.



Stacking Multiple Electropherograms

Electropherogram data for multiple capillaries can be stacked vertically in the graph pane for comparison. To do this:

1. Click **Single View**.
2. Select multiple rows in the experiment pane.
3. Click the **Stack the Plots** button. The individual electropherograms for each row selected will stack in the graph pane.

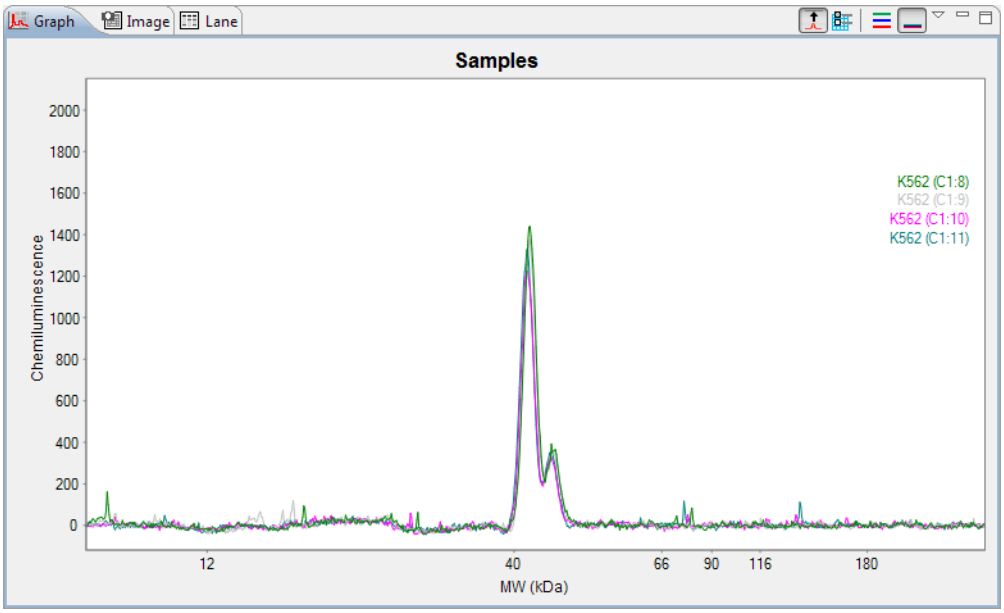


Users can customize the colors used for the stacked plot display. For more information see “Selecting Custom Plot Colors for Graph Overlay” on page 392.

Overlaying Multiple Electropherograms

The electropherogram data for multiple capillaries can also be overlaid on top of each other for comparison in the graph pane. To do this:

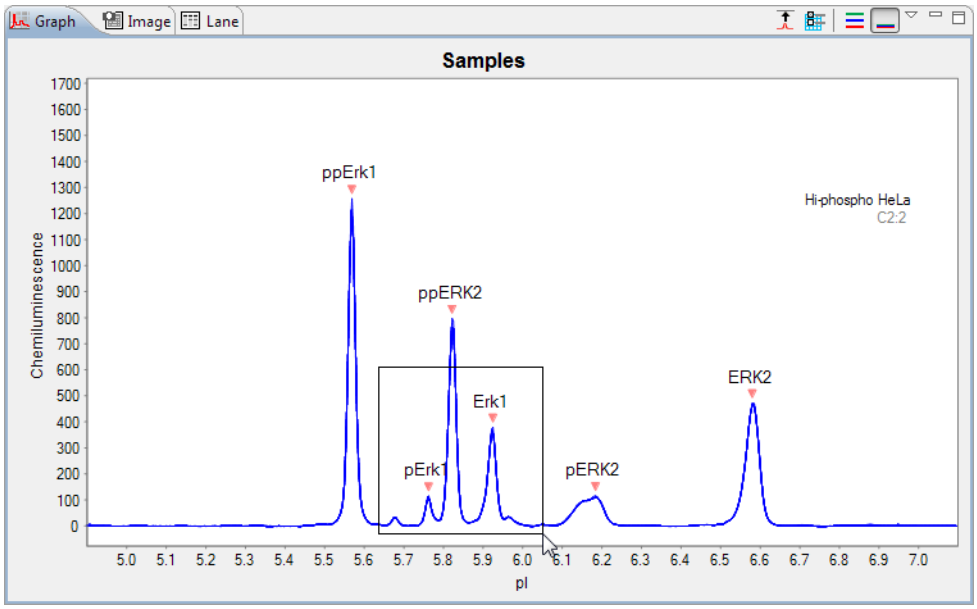
1. Click **Single View**.
2. Select multiple rows in the experiment pane.
3. Click the **Overlay the Plots** button. The individual electropherograms for each row selected will overlay in the graph pane.



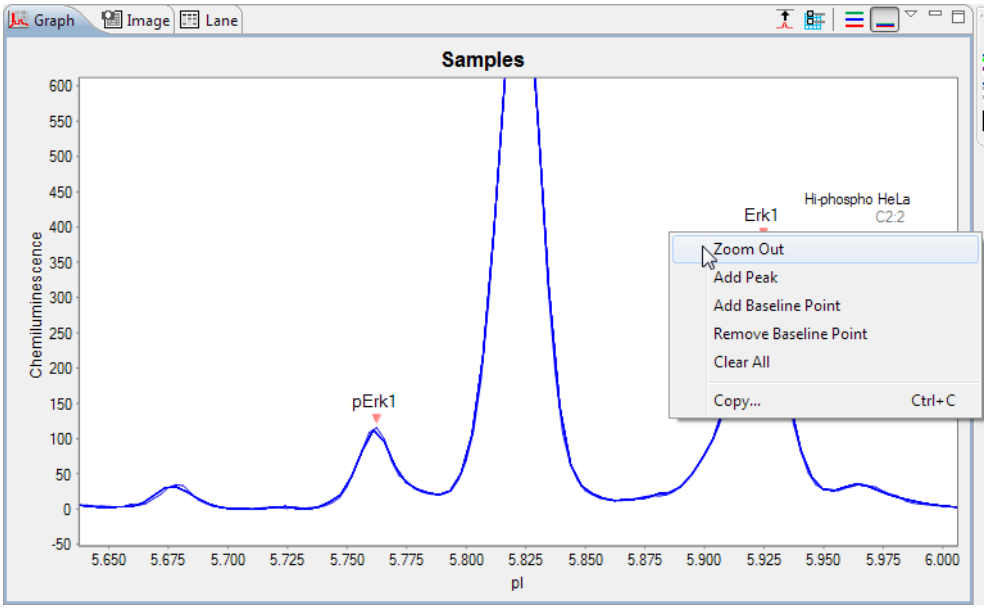
Users can customize the colors used for the overlay plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 392.

Zooming

To zoom in on a specific area of the electropherogram, hold the mouse button down and draw a box around the area with the mouse:

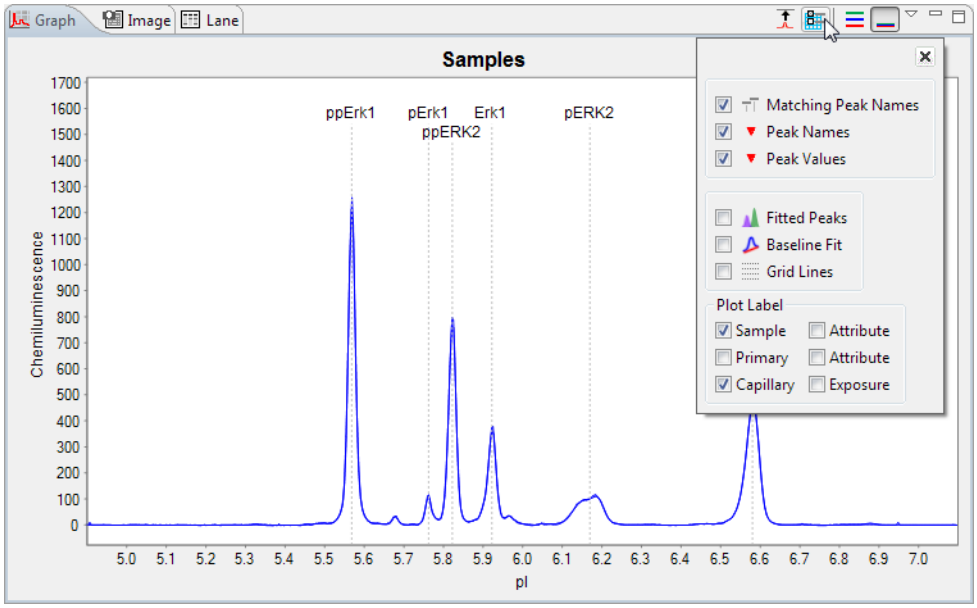


To return to default scaling, right click in the electropherogram and click **Zoom Out**.



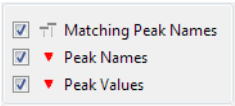
Customizing the Data Display

Electropherogram peak labels, plot labels and display options can be customized by the user. To do this, select the **Graph Options** button.

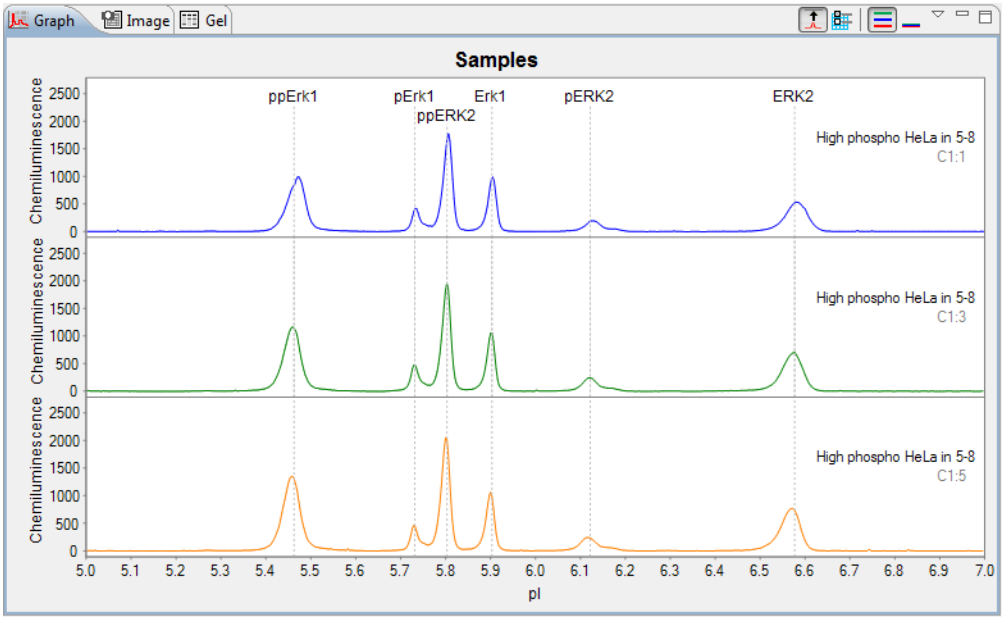


Peak Labels

You can customize the labels used to identify peaks in the electropherogram with these options:

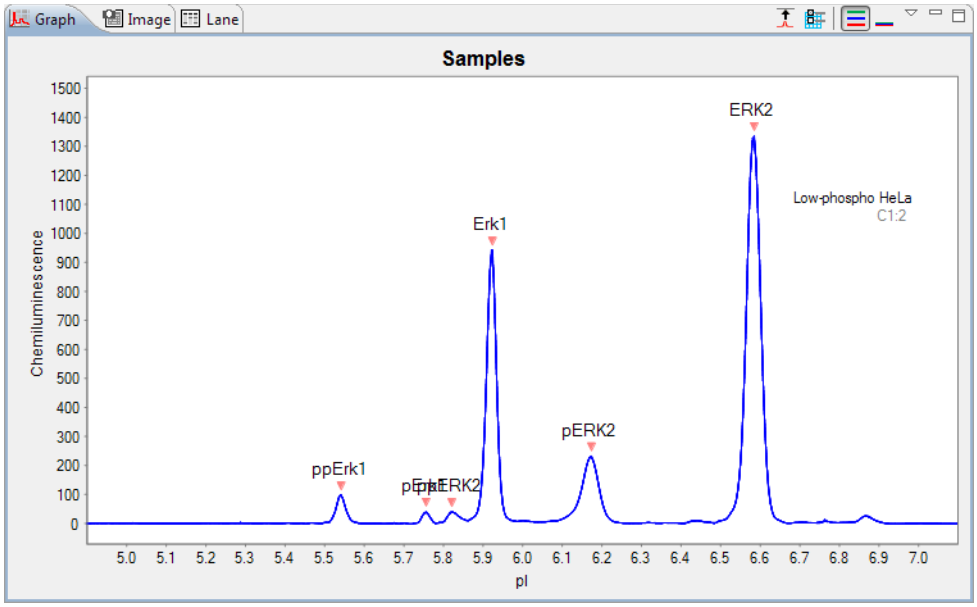


Matching Peak Names - Checking this box will draw vertical lines through each named peak. Using this option with Stack the Plots or Overlay the Plots features is useful for visual comparison of named peaks across multiple capillaries.



- **Peak Names** - Checking this box will display peak name labels on all named peaks in the electropherogram.

NOTE: If more than one peak label option is selected, peak name labels will always be used for named peaks.

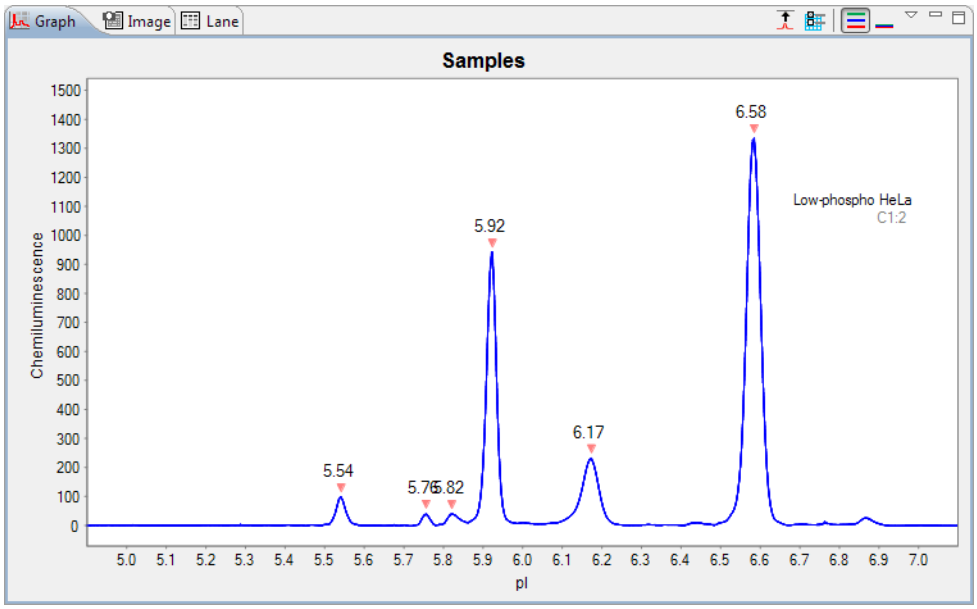


- **Peak Values** - Checking this box will display the pI labels on all peaks in the electropherogram.

NOTES:
When viewing standards and registration data, this option is called Peak Positions. Labels displayed are peak positions rather than pI.

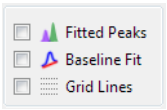
If more than one peak label option is selected, peak name labels will always be used for named peaks.

The reported pI for immunodetected sample proteins in Compass may vary slightly from predicted pIs based on sample, buffer and assay conditions.



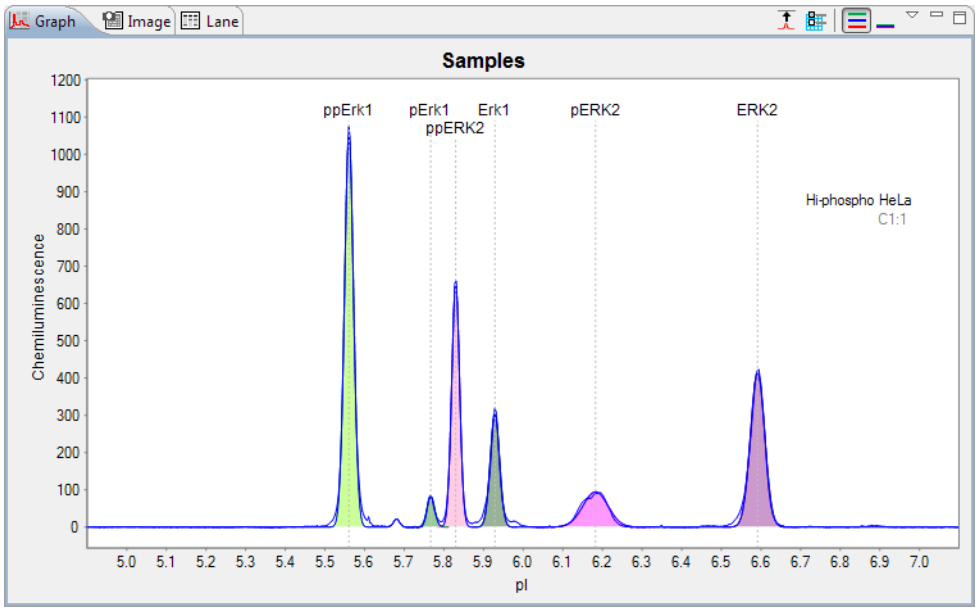
Baseline and Grid Options

You can view the calculated baseline fit, peak integration and show grid lines with these options.



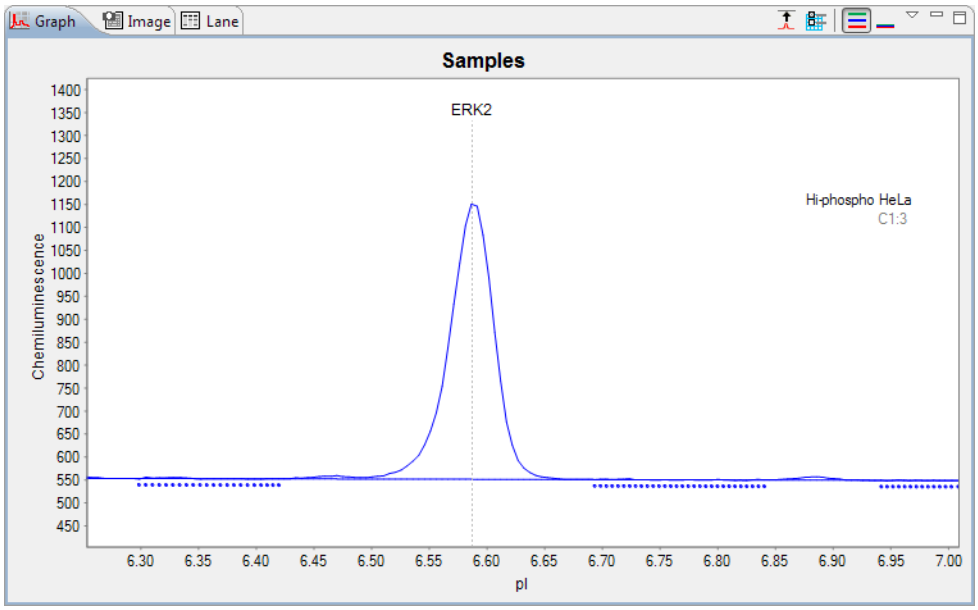
- **Fitted peaks** - Checking this box will display how the peaks were fit by the software.

NOTE: This option is only available for sample data.

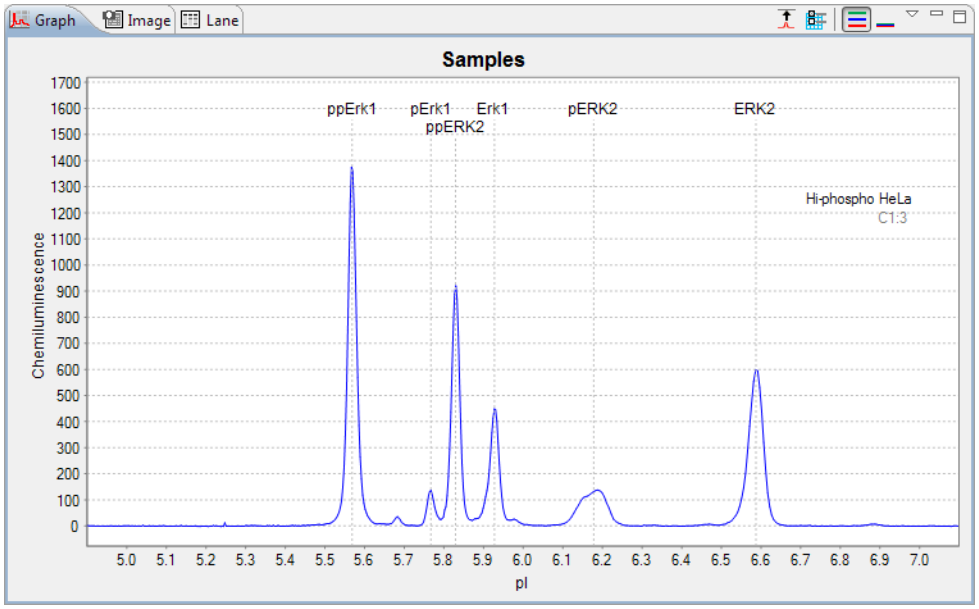


- **Baseline Fit** - Checking this box will display the calculated baseline for the peaks. Baseline points will also display for regions of the electropherogram considered to be at baseline.

NOTE: This option is only available for sample data.

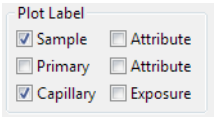


- **Grid Lines** - Checking this box will display grid lines in the graph area.



Plot Labels

You can customize the plot labels displayed on the electropherogram with these options.

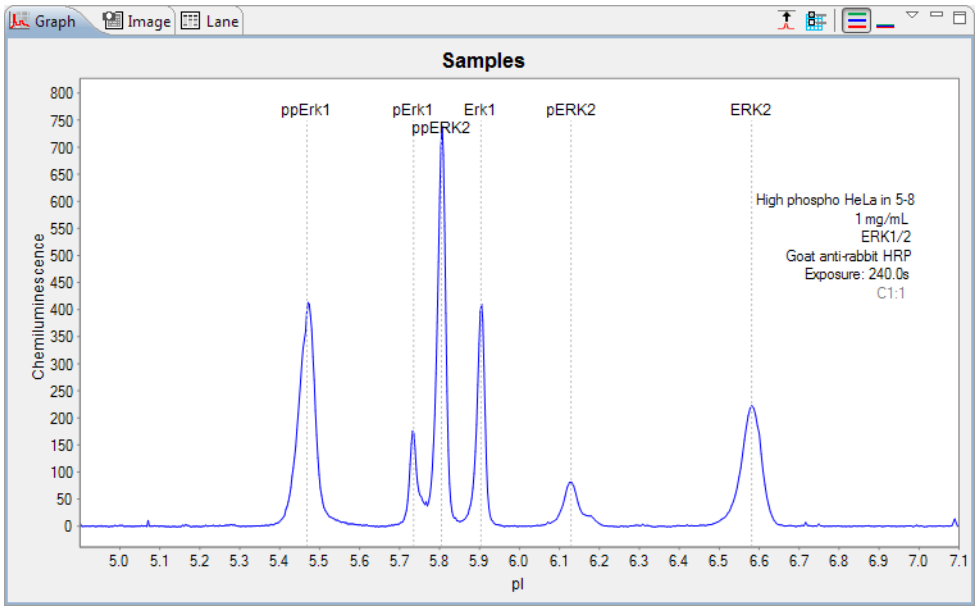


Plot labels are shown on the right side of the graph pane.

- **Sample** - Checking this box will display the sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Checking this box will display the primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** - Checking this box will display the cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
- **Attributes** - Checking this box will display attribute text. If attribute information was entered for samples or primary antibodies in the assay template (Assay screen), they can be selected as plot labels.

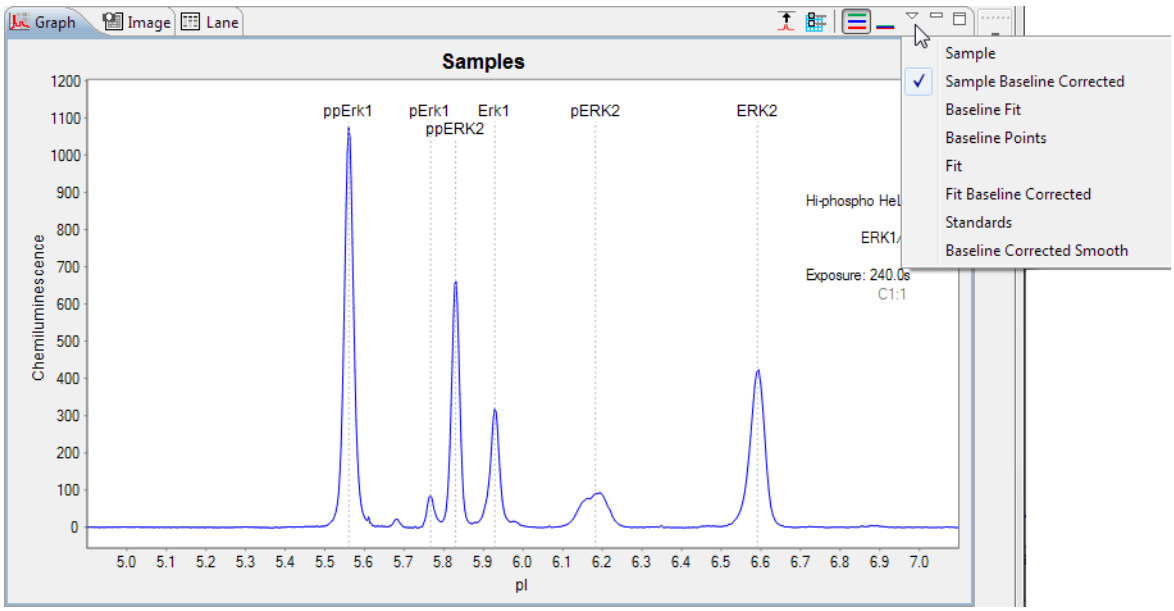
- **Exposure** - Checking this box will display the exposure time(s) used for the data.

The following example shows an electropherogram with all plot labels selected:



Selecting Data Viewing Options

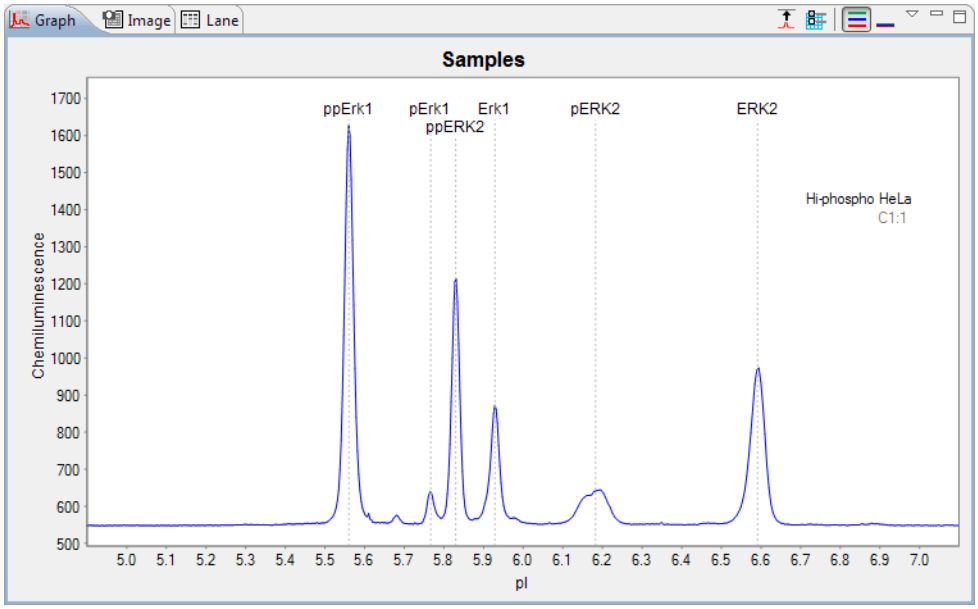
The graph view menu provides multiple user options for changing what type of electropherogram data is displayed. To access the view menu, click the down arrow next to the graph pane toolbar:



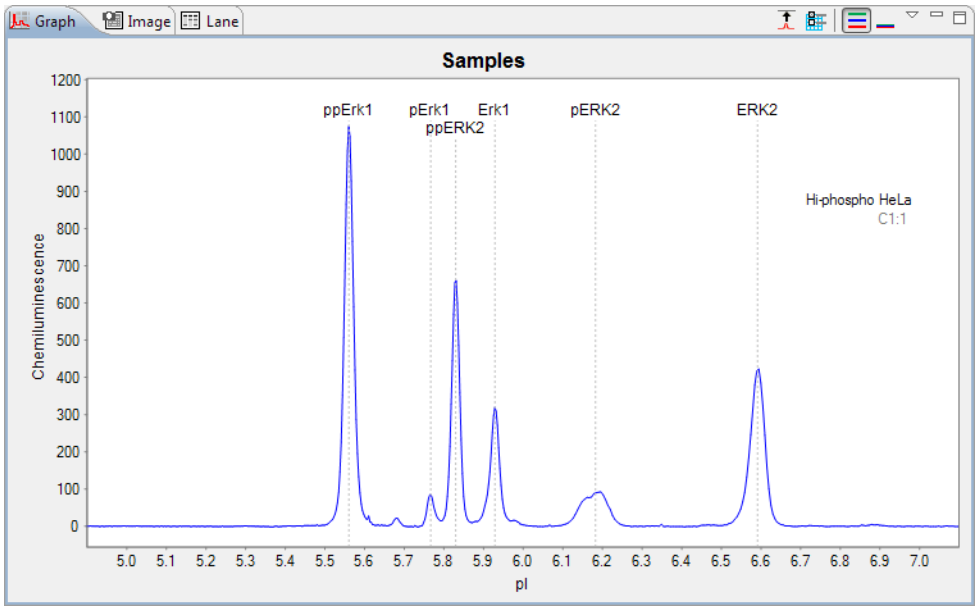
A check mark next to the menu option indicates it is currently selected. Multiple options can be selected at a time.

NOTE: Unless otherwise noted, graph view menu options are available for sample data only.

- **Sample** - Clicking this option will display raw, uncorrected sample data.

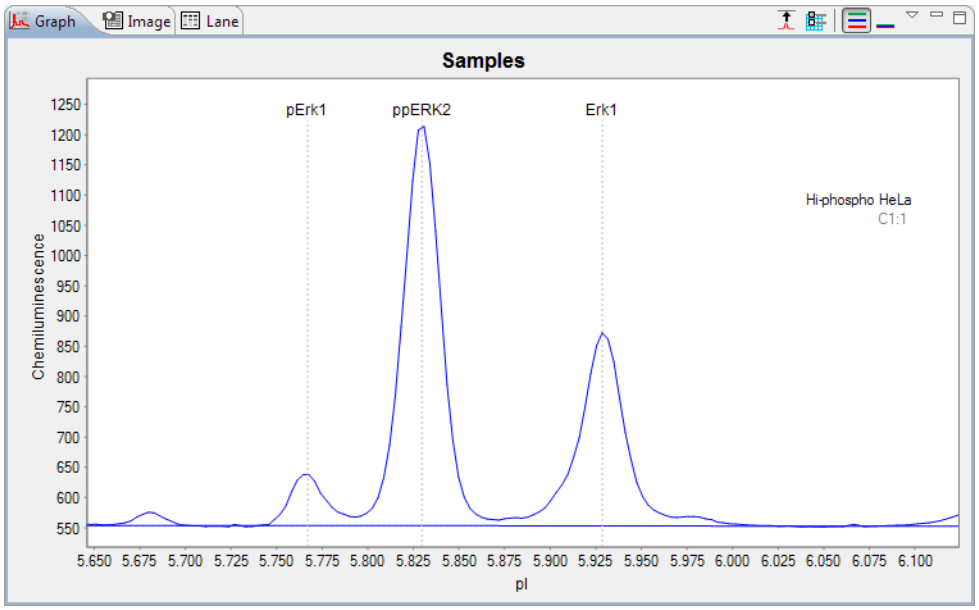


- **Sample Baseline Corrected** - Clicking this option will display sample data with the baseline subtracted (zeroed). This is the default view.



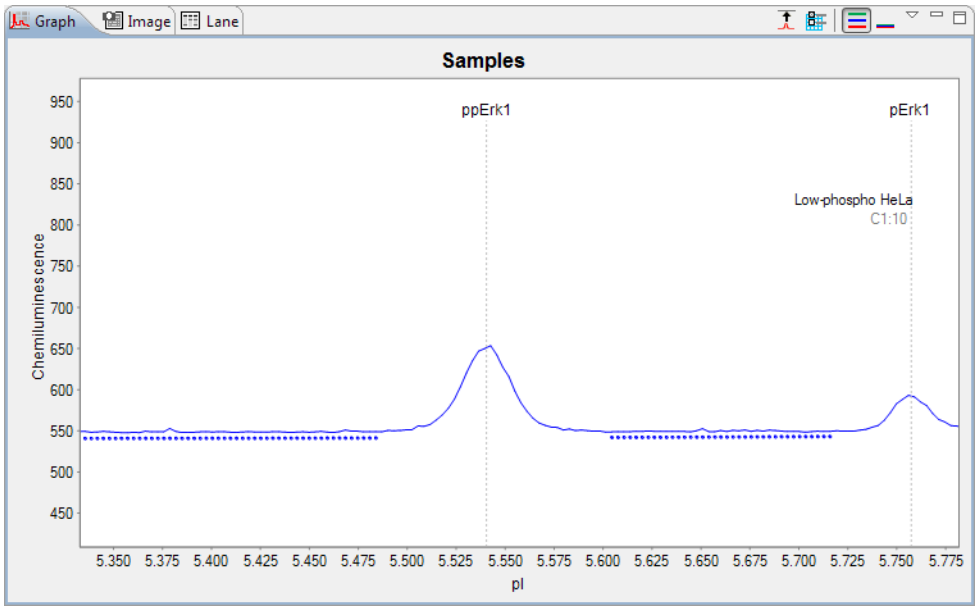
- **Baseline Fit** - Clicking this option will display the calculated baseline for the raw sample data. In the example that follows, both Baseline fit and Sample are selected.

NOTE: This option is selected automatically when Baseline Fit is selected in graph options.

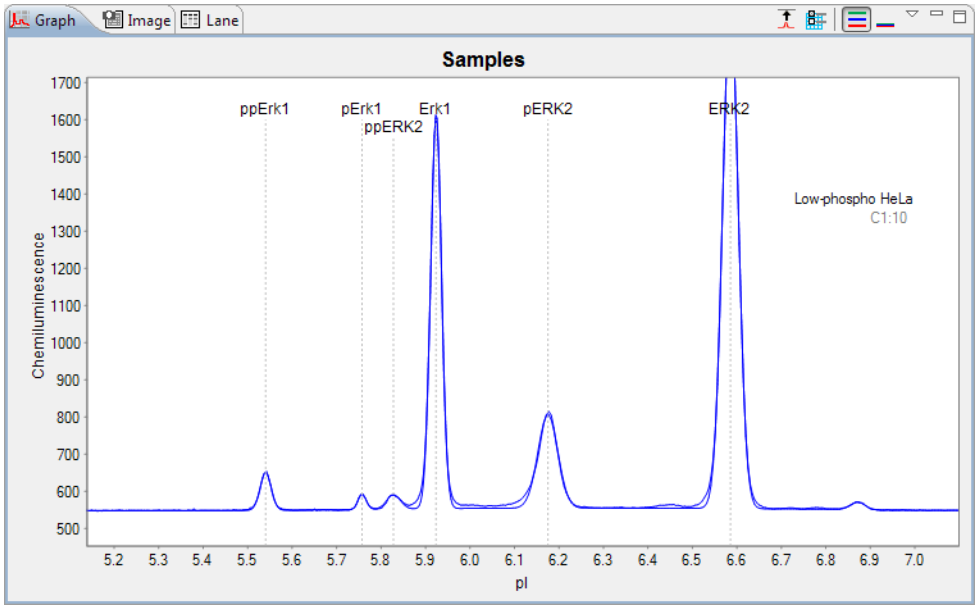


- **Baseline Points** - Clicking this option will display regions of the electropherogram considered to be at baseline. In the example that follows, both Baseline Points and Sample are selected.

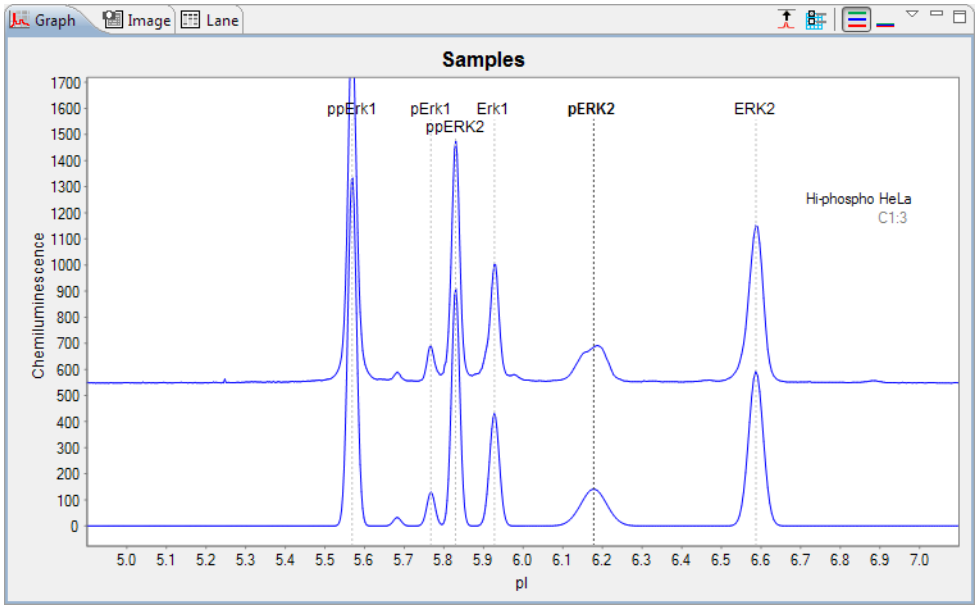
NOTE: This option is selected automatically when Baseline Fit is selected in graph options.



- **Fit** - Clicking this option will display the bounding envelope of the fitted peaks as calculated by the software for the raw sample data. In the example that follows, both Fit and Sample are selected.

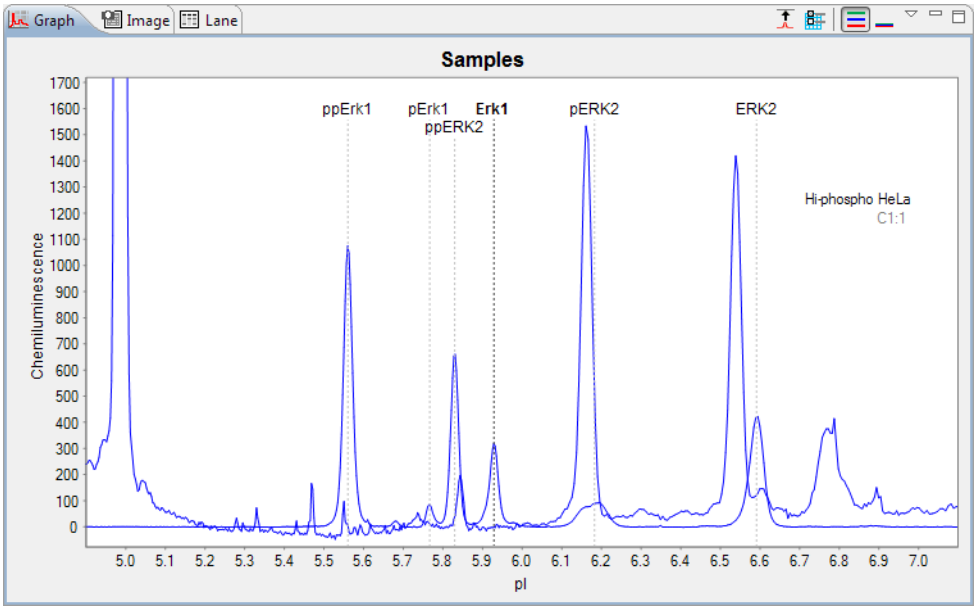


- **Fit Baseline Corrected** - Clicking this option will display the fitted peaks as calculated by the software for the sample baseline corrected data. In the example that follows, both Fit Baseline Corrected and Sample are selected, the fit plot is on the bottom.



NOTE: When viewing standards or registration data, this option is called Standards Fluorescence or Registration Fluorescence, respectively.

- **Standards** - Checking this box aligns the pI of the raw standards data to the sample data and overlays both electropherograms in the graph pane.

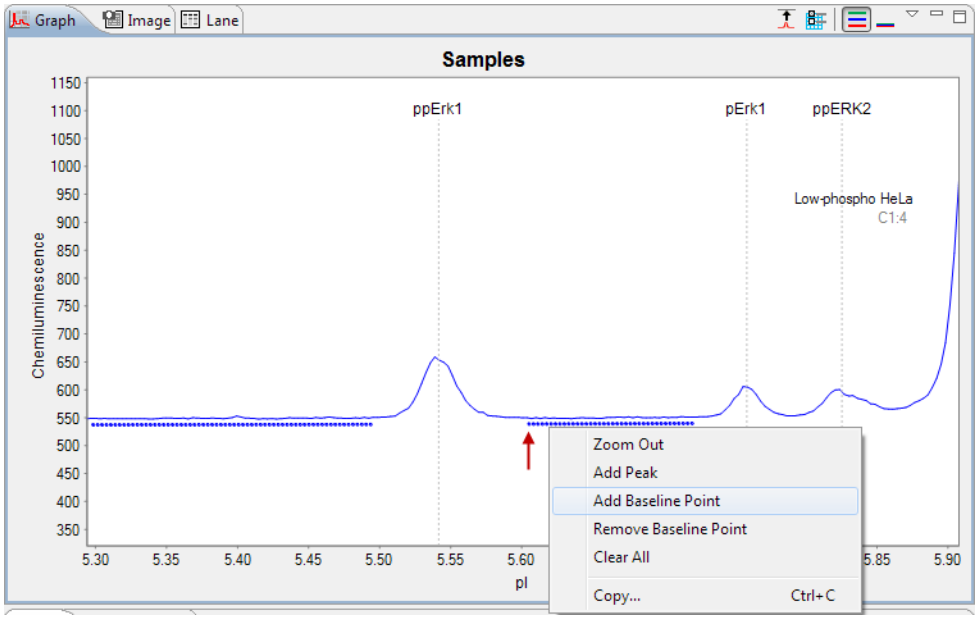


Adding and Removing Baseline Points

Points in the baseline can be added or removed as needed. To do this:

1. Click the **Graph Options** button in the graph pane toolbar and check **Baseline Fit**. This will display baseline points for the raw sample data.
2. Use the mouse to draw a box around the area you want to correct. This will zoom in on the area.

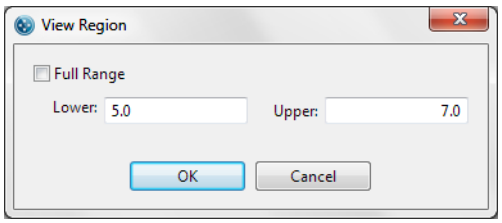
3. Right click a baseline point and click **Add Baseline Point** or **Remove Baseline Point**.



*NOTE: To clear the manual addition or removal of baseline points and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

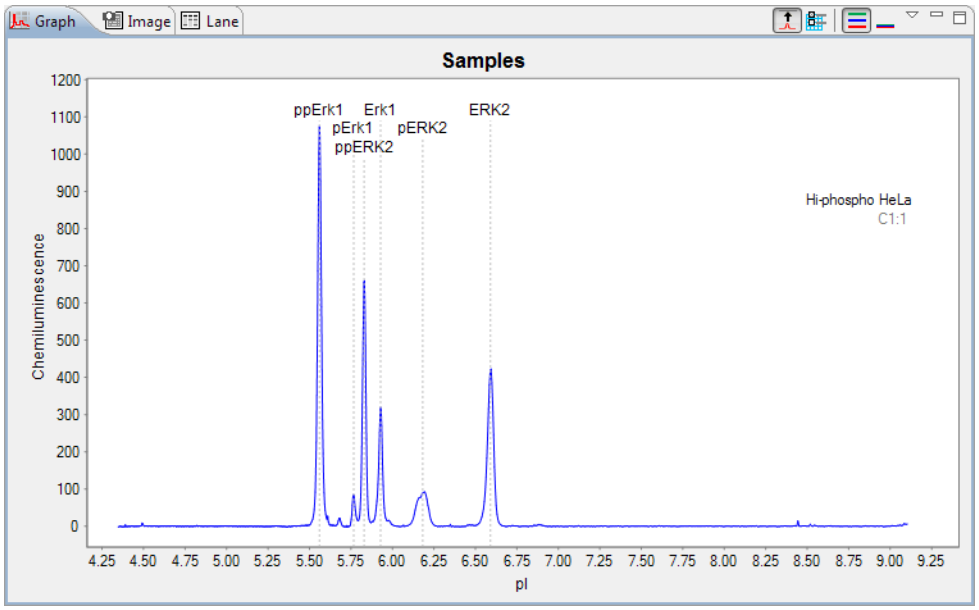
Selecting the X-Axis pI Range

The pI range used for the x-axis can be changed. To do this, Select **View** in the main menu and click **View Region**. The following pop-up window will display:



- **To change the x-axis pI range displayed for the run data** - Enter new values in the Lower or Upper range in pI and click **OK**. Electropherogram and virtual blot data will update to display only the data in the entered range.

- **To see the full x-axis pI range included in the run data** - Check **Full Range**. Electropherogram and virtual blot data will update to display the full range of available data.



NOTE: You can change the default x-axis range that Compass uses. For more information, see "Peak Fit Analysis Settings" on page 360.

Closing Run Files

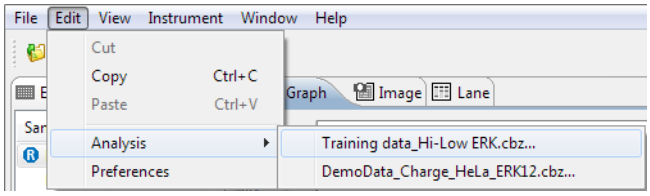
If more than one run file is open, you can close just one file or all the open files at the same time.

- **To close one of multiple open run files** - In the experiment pane, click on one of the sample rows in the file. Next click **File** from the main menu and click **Close**.
- **To close all open run files** - Select **File** from the main menu and click **Close All**.

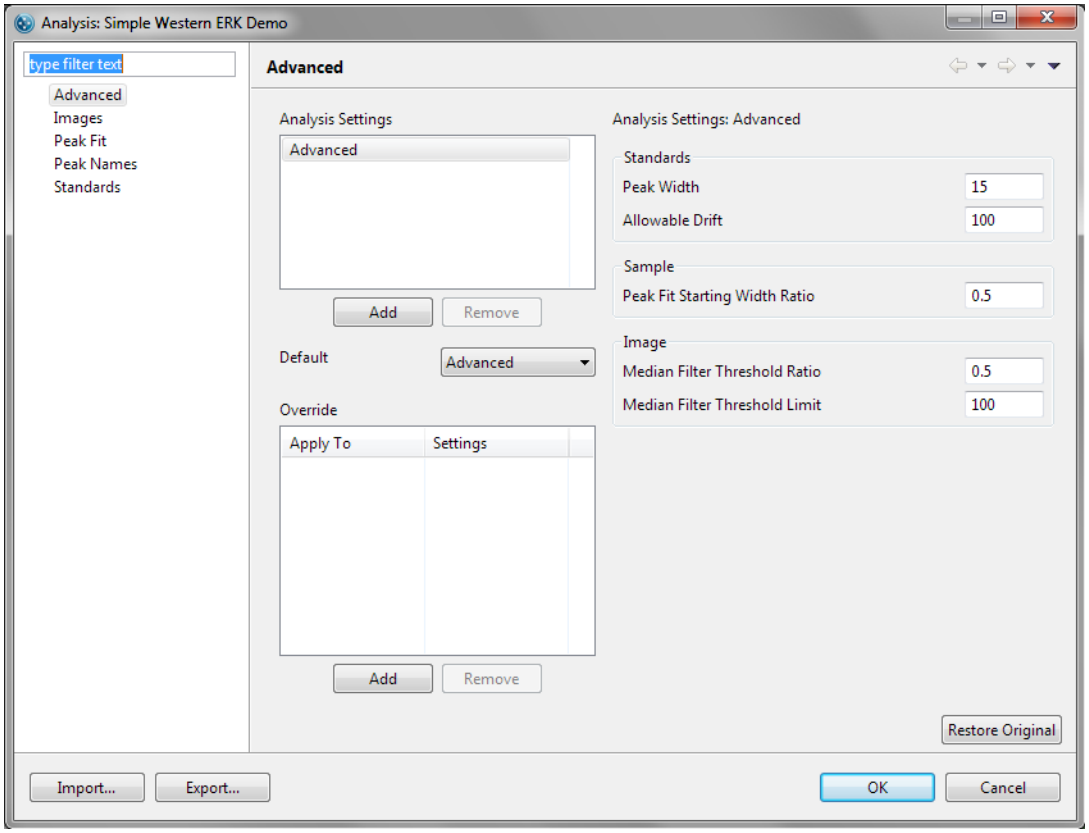
Compass Analysis Settings Overview

Compass has a variety of analysis features and settings that users can modify as needed to enhance run data.

To access these settings, select **Edit** in the main menu and click **Analysis**. If more than one run file is open, select the run file you want to view analysis settings for from the list:



The following screen will display:



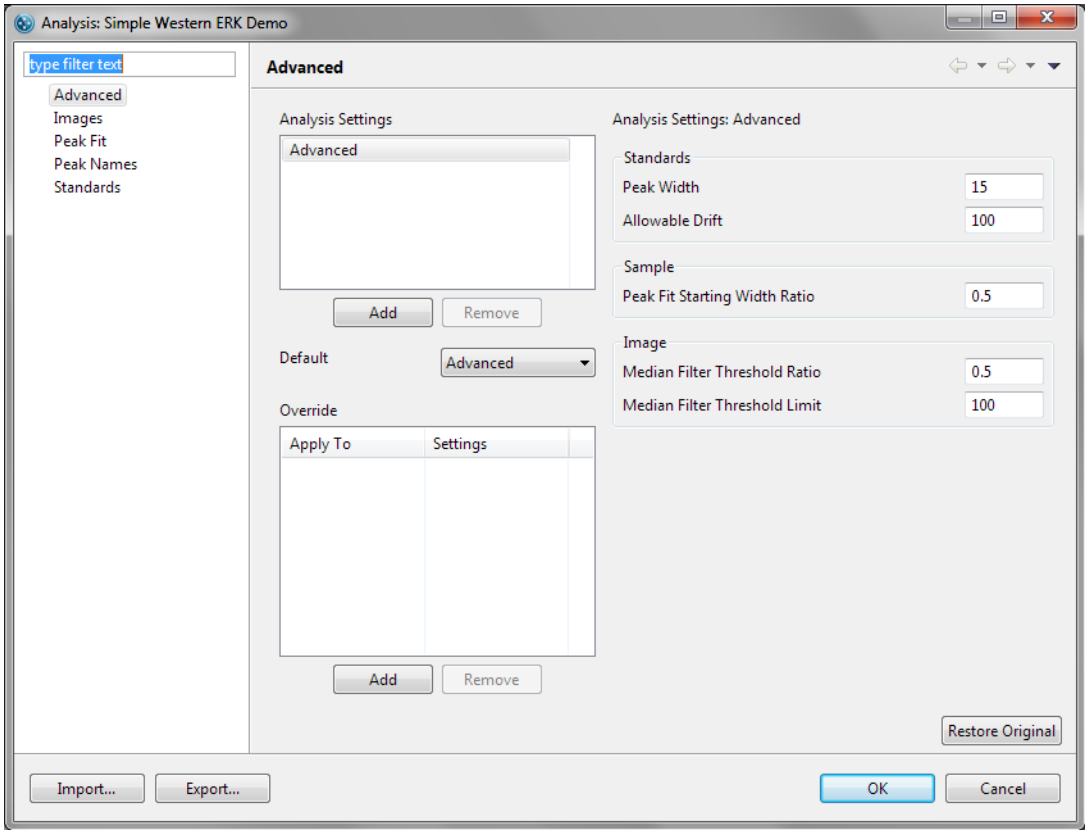
To move between analysis pages in this window, click on an option in the list on the left. The following analysis settings can be user-customized in Compass:

- **Advanced** - Lets you customize analysis settings for samples, standards and image data.
- **Images** - Lets you view the chemiluminescent exposures taken during the run and view data for different exposures in the Analysis screen.
- **Peak Fit** - Lets you customize peak fit settings for sample data.
- **Peak Names** - Lets you enter custom naming settings for sample proteins associated with specific primary antibodies or attributes and have Compass automatically label the peaks in the run data.
- **Standards** - Lets you customize the pI and positions Compass uses to identify fluorescent standards and registration peaks.

Advanced Analysis Settings

The advanced analysis settings page lets you view and change analysis settings for samples, standards and image data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 386.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 387.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Standards Settings

- **Peak Width** - The approximate width (at full width half max) used to filter out fluorescence artifacts which improves recognition of standards. The default value is 15.
- **Allowable Drift** - The distance in pixels that standards are expected to move compared to their entered positions in the standards analysis page. This setting assists in recognition of standards. The default value is 100.

Sample Settings

- **Peak Fit Starting Width Ratio -** Focuses the peak fit towards the peak center and aids the overall peak fitting. The default value is 0.5.

Image Settings

- **Median Filter Threshold Ratio** - Pixel ratio used to filter out camera artifacts. The default value is 0.5.
- **Median Filter Threshold Limit** - Pixel threshold value used to filter out camera artifacts. The default value is 100.

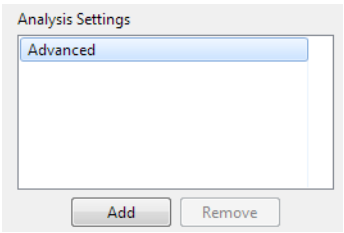
Advanced Analysis Settings Groups

Advanced analysis settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
ProteinSimple recommends using the Compass default values for advanced analysis settings. These settings are included in the default Advanced group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 386.

Analysis groups are displayed in the analysis settings box:

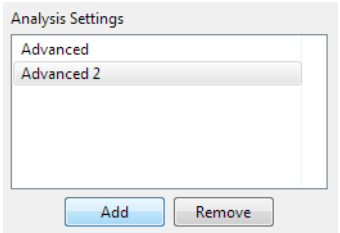


The Advanced group shown contains the Compass default analysis settings. Users can make changes to this group and create new groups.

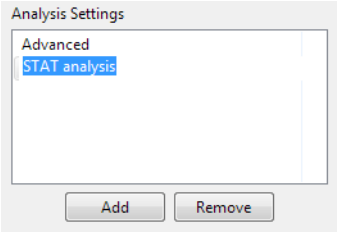
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Analysis Group

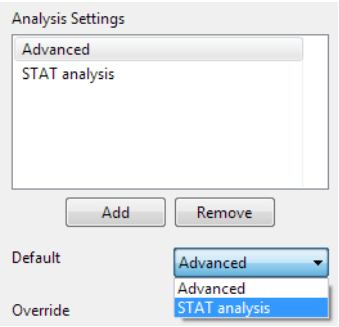
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



3. Click on the new group and enter a new name.



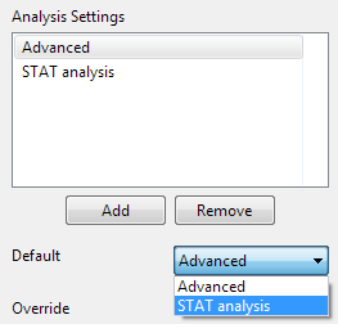
4. Modify standards, sample or image parameters as needed.
5. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.



6. Click **OK** to save changes.

Changing the Default Analysis Group

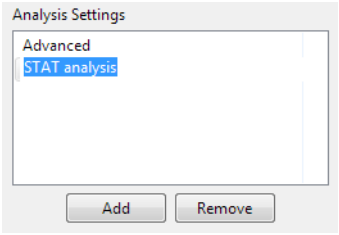
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying an Analysis Group

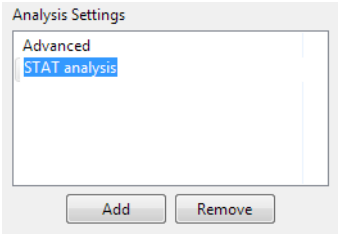
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify standards, sample or image parameters as needed.
4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

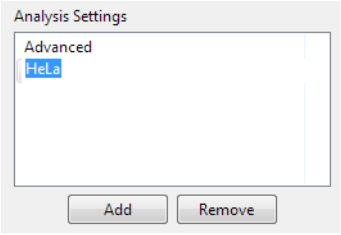
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



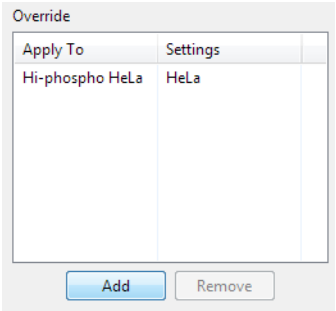
3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

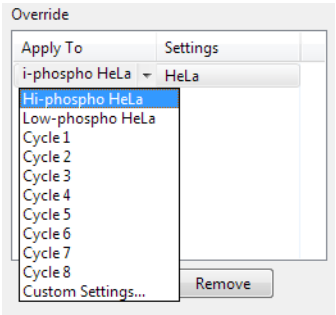
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.



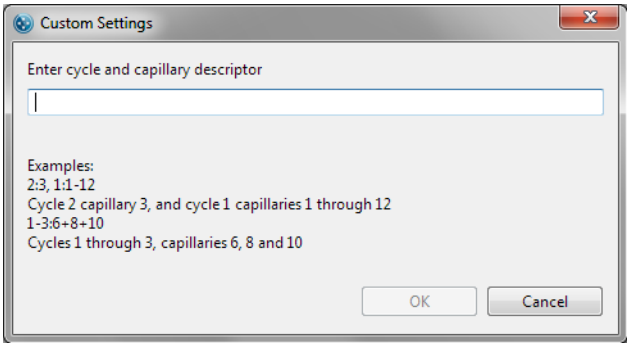
4. Click the cell in the **Apply To** column, then click the down arrow.



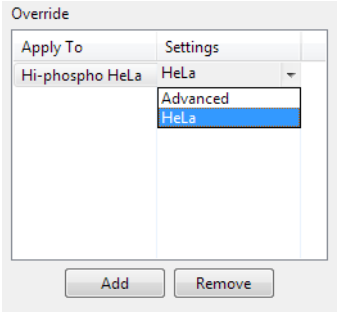
5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.

- **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



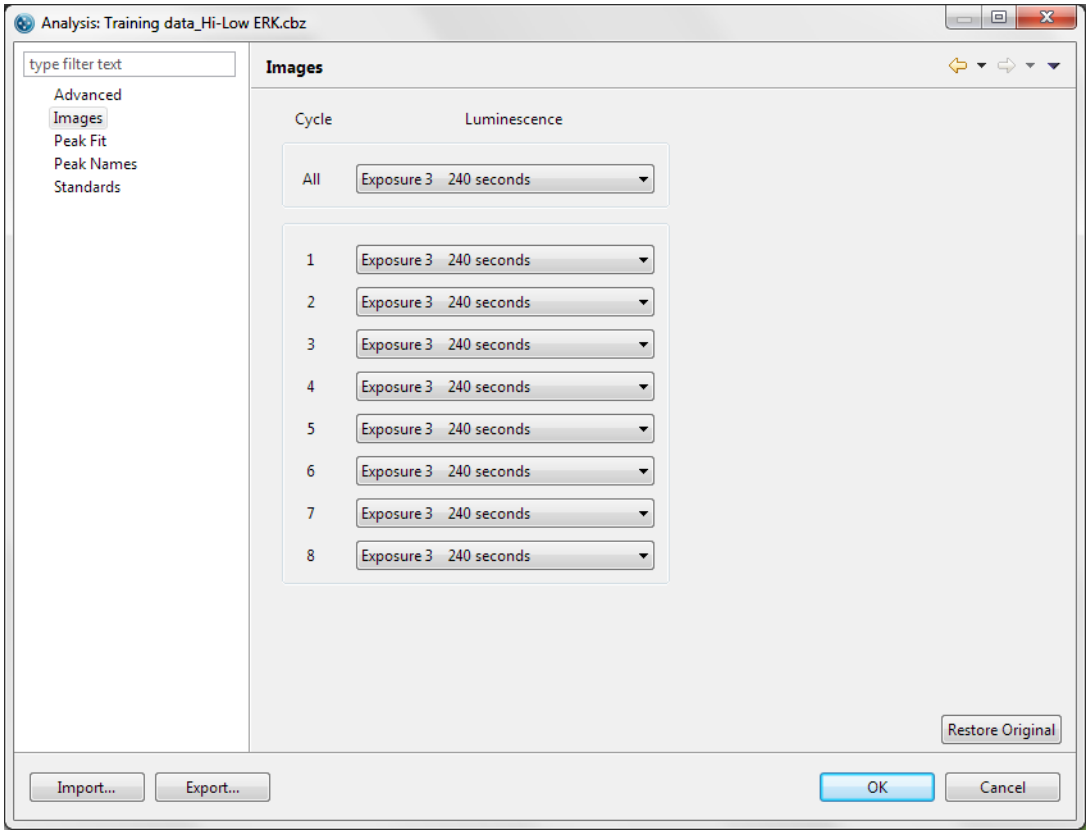
6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Images Analysis Settings

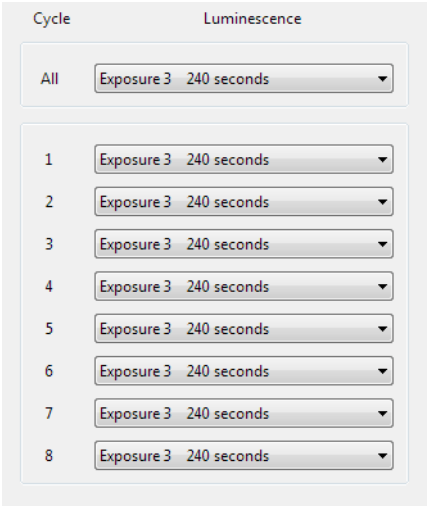
The Images analysis settings page lets you see what chemiluminescent exposures were taken during the run, and view data for different exposures in the Analysis screen. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 386.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 387.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Exposure Settings

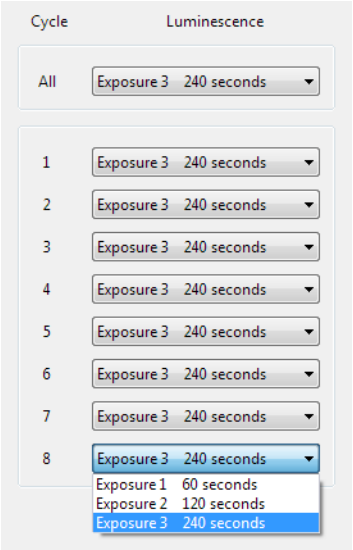
The exposure used for the sample data displayed in the Analysis screen is shown in the All box:



NOTE: Peggy runs up to eight cycles (12 capillaries per cycle). The individual exposure setting selected for a specific cycle is applied for all 12 capillaries in that cycle unless selected for All cycles.

- **Exposure #** - Sample data displayed in the Analysis screen is for this specific exposure only.

To see the number of exposures and exposure times used for the run data, click the arrow in the drop down list next to **All**.

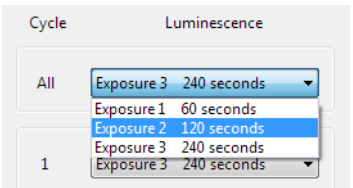


NOTE: The number of exposures taken and exposure times shown were specified in the assay protocol (Assay screen) prior to executing the run and cannot be modified.

Changing the Sample Data Exposure Displayed

The exposure used for the sample data displayed in the Analysis screen can be changed. To do this:

1. Select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.
2. Click the arrow in the drop down list next to **All** and select an exposure setting:

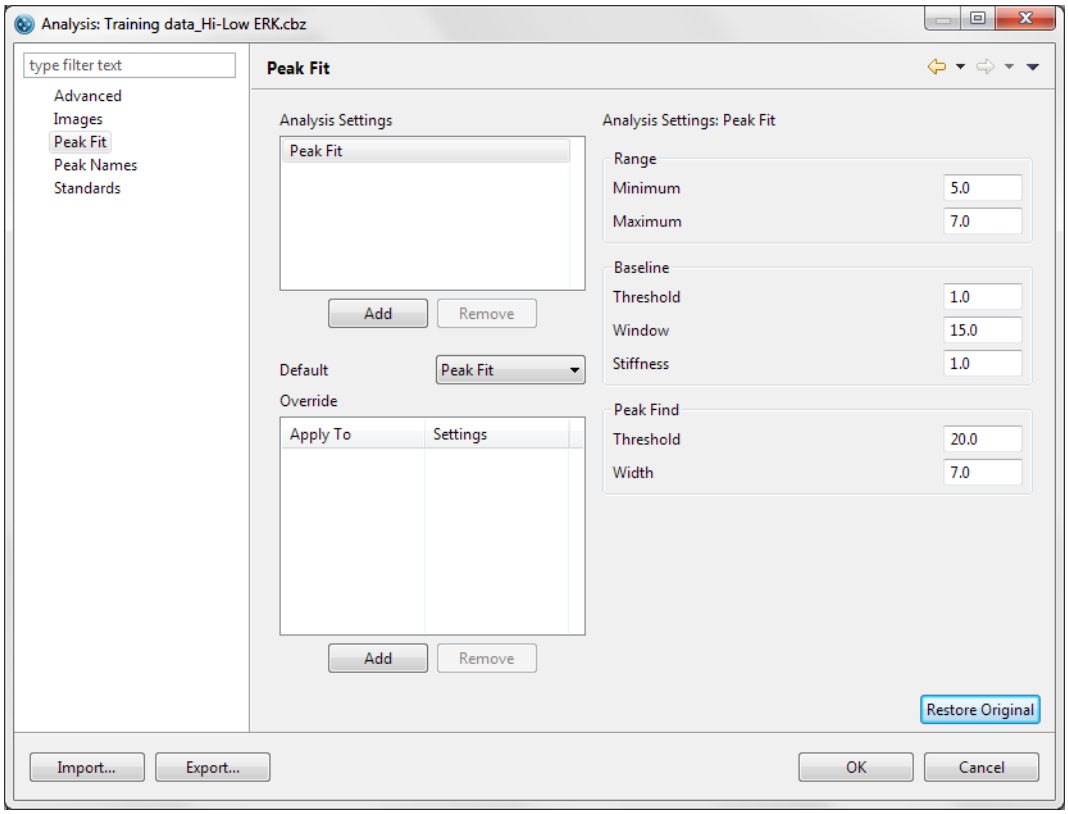


3. Click **OK** to save changes and exit. Sample data for the exposure selected will display in the Analysis screen.

Peak Fit Analysis Settings

The peak fit analysis settings page lets you view and change peak fit settings for sample data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 386.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 387.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Range Settings

- **Minimum** - The pI value below which peaks will not be identified. This value will also be used as the default lower pI range for the data displayed in the electropherogram and virtual blot. The default value is 5.
- **Maximum**: The pI value above which peaks will not be identified. This value will also be used as the default upper pI range for the data displayed in the electropherogram and virtual blot. The default value is 7.

Baseline Settings

- **Threshold** - The variance, or roughness, in a baseline data segment below which a point will be called part of the baseline. The default value is 1.0.
- **Window** - How long baseline data segments are expected to be in pixels. Shorter segments will allow the baseline to follow plateau sections of the signal. The default value is 15.
- **Stiffness** - The amount the baseline is allowed to vary from a straight line. Settings between 0.1 and 1.0 make the baseline fit closer to a straight line. Settings from 1.0 to 10.0 will make the baseline fit follow the data more closely. The default value is 1.0.

Peak Find Settings

- **Threshold** - The minimum signal to noise ratio required for a peak to be identified. A setting of 1.0 will detect many peaks, a setting of 30.0 will detect fewer peaks. The default value is 20.0.
- **Width** - The approximate peak width (at full width half max) in pixels used to detect peaks. The minimum value for this setting is 3.0. Larger widths help to eliminate the detection of shoulder peaks and noise peaks. The default value is 7.0.

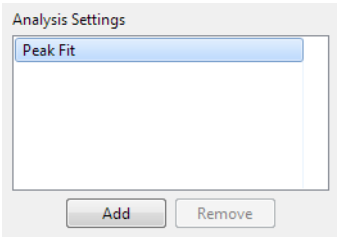
Peak Fit Analysis Settings Groups

Peak fit settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
ProteinSimple recommends using the Compass default values for peak fit analysis settings. These settings are included in the default Peak Fit group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 386.

Peak fit groups are displayed in the analysis settings box:

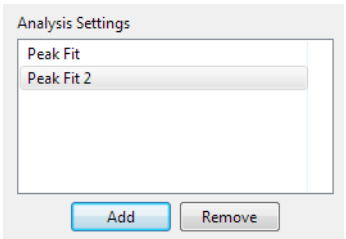


The Peak Fit group shown contains the Compass default analysis settings. Users can make changes to this group and create new groups.

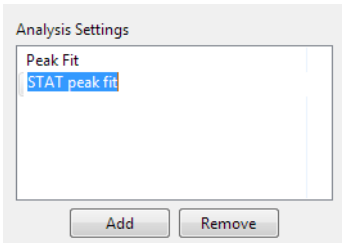
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Peak Fit Group

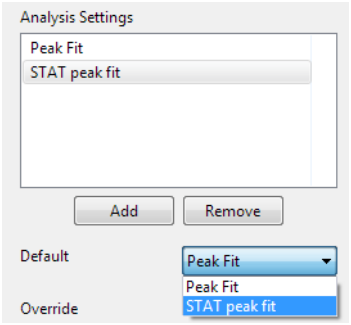
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



3. Click on the new group and enter a new name.



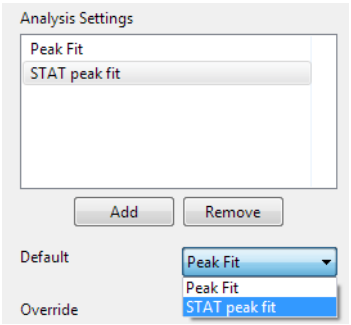
4. Modify range, baseline or peak find parameters as needed.
5. To use the new group as the default peak fit settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Peak fit settings in the new group will then be applied to the run data.



6. Click **OK** to save changes.

Changing the Default Peak Fit Group

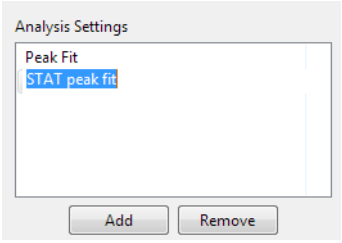
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Peak fit settings in the group selected will be applied to the run data.

Modifying a Peak Fit Group

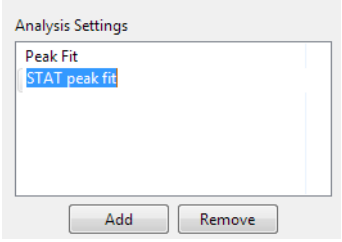
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify range, baseline or peak find parameters as needed.
4. Click **OK** to save changes. The new peak fit settings will be applied to the run data.

Deleting a Peak Fit Group

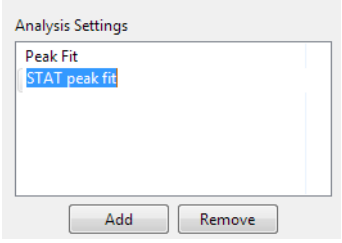
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



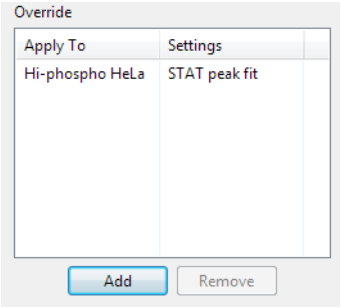
3. Click **OK** to save changes.

Applying Peak Fit Groups to Specific Run Data

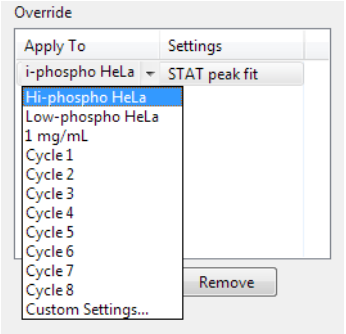
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of peak fit groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

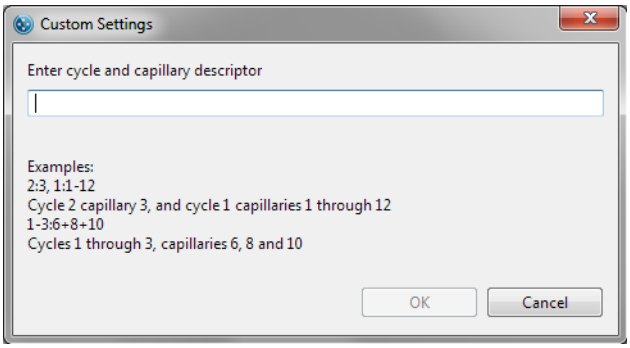


4. Click the cell in the **Apply To** column, then click the down arrow.

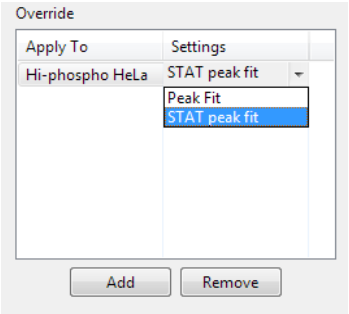


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the peak fit group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

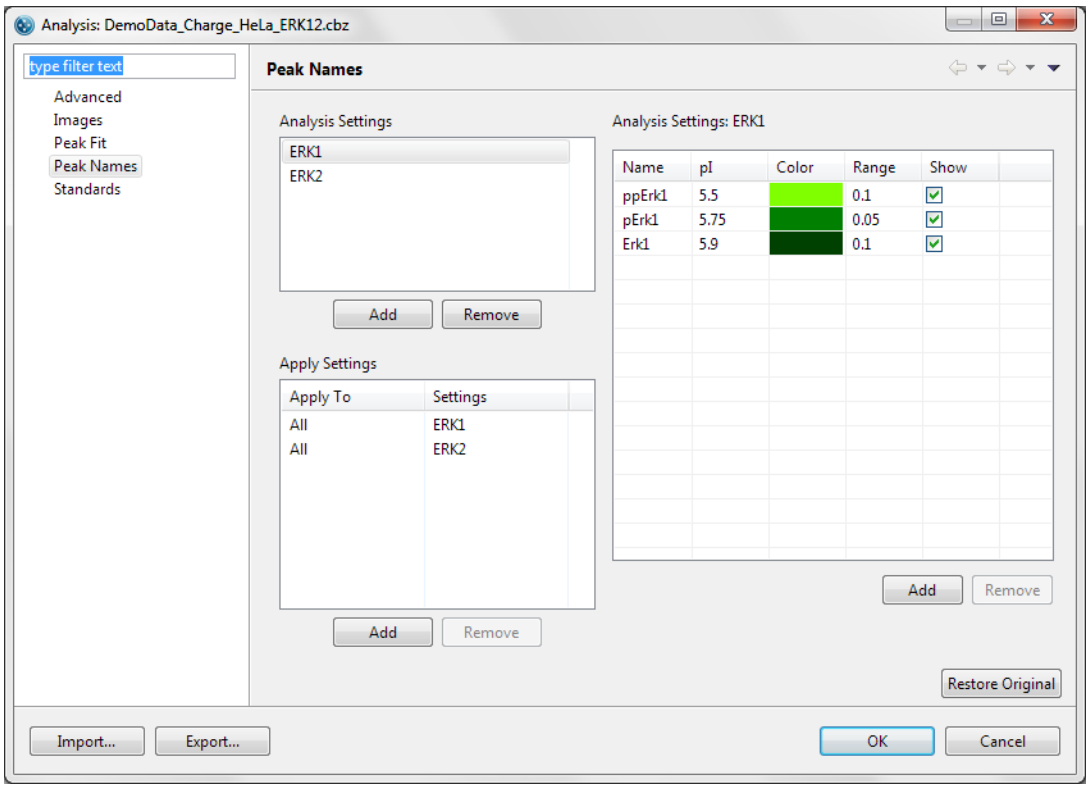


7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Peak Names Settings

The peak names analysis settings page lets you enter custom naming settings for sample proteins. Compass can automatically label sample peaks in the run data associated with specific blocking reagent names, primary antibody names or attributes. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 386.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 387.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.

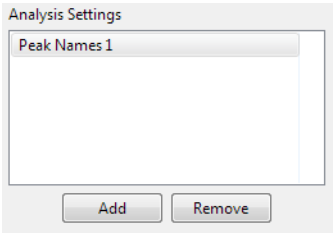
- Click **Cancel** to exit without saving changes.

Peak Names Analysis Settings Groups

Peak name settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, blocking reagent names or primary antibody names and attributes in the run data.

NOTE: Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 386.

Peak name groups are displayed in the analysis settings box:

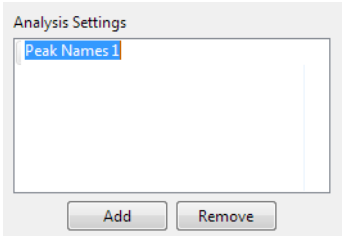


The Peak Names group shown is a Compass template group. Users can make changes to this group and create new groups.

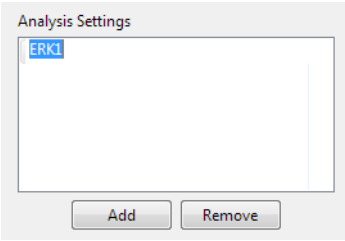
To view settings for a group, click on the group name in the analysis settings box.

Creating a Peak Names Group

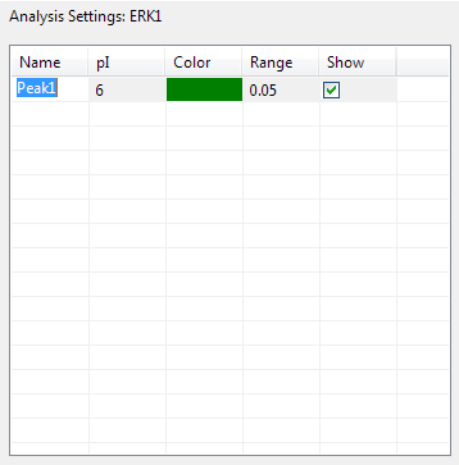
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the **Peak Names 1** template group in the analysis settings box.



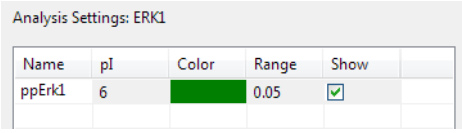
3. Enter a new name for the group.



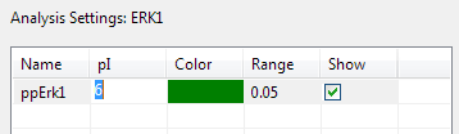
4. Click in the first cell in the **Name** column in the analysis settings peak table.



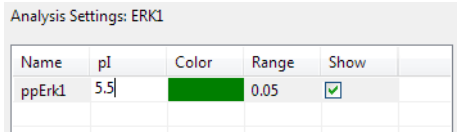
5. Enter a sample protein name associated with the primary antibody used in the run.



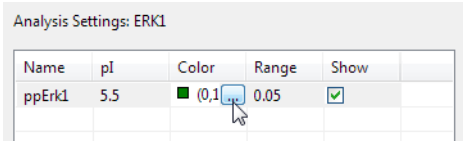
6. Click in the first cell in the **pI** column.



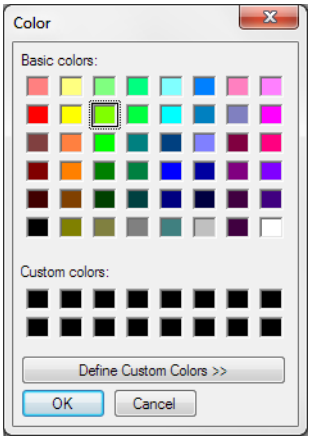
7. Enter the pI for the sample protein.



8. Click in the first cell in the **Color** column, then click the button.

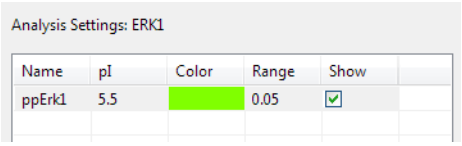


The color selection box will display:

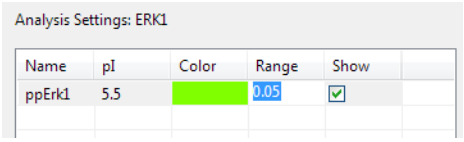


The color selected will be used to identify the sample protein peak in the peaks and capillaries table in the Analysis screen.

9. Click a color or define a custom color and click **OK**. The color selection will update in the table:



10. Click in the first cell in the **Range (%)** column.



11. Enter a range window for the pI entered. Compass will automatically name peaks found within this percent of the pI. For example, if the pI entered is 5.5 and a 0.1 pI range is used, all peaks between pI 5.4 and 5.6 will be identified with this peak name.

NOTE: The reported pI for immunodetected sample proteins in Compass may vary slightly from predicted pIs based on sample, buffer and assay conditions.

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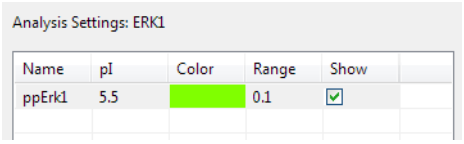
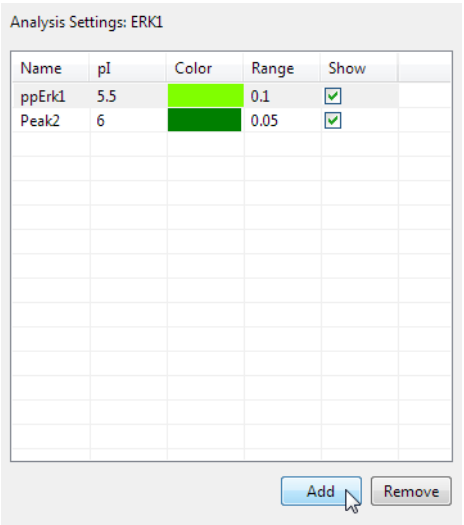
12. Select the checkbox in the first cell of the **Show** column. This will turn peak naming on for the sample protein.

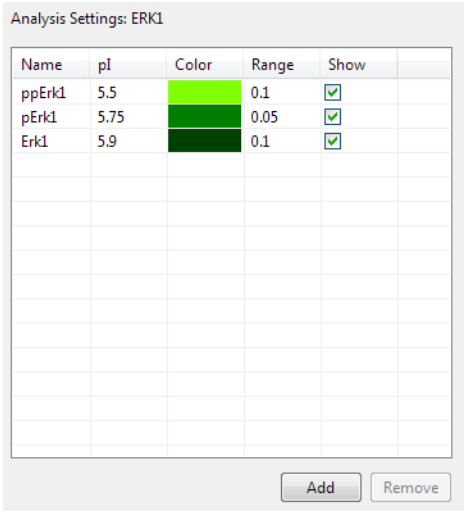


To turn peak naming off for a particular sample protein, deselect the checkbox in the Show column.

13. To add another sample protein, click **Add** under the analysis settings peak table:



14. Repeat the previous steps to enter information for other sample proteins. In the following example, three sample proteins were entered:

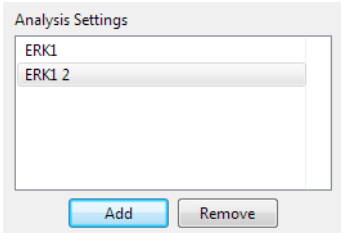


To remove a sample protein, select its row and click **Remove**.

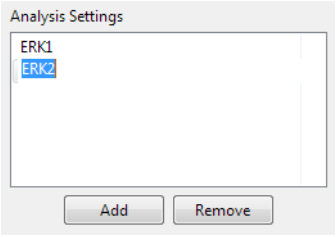
15. Click **OK** to save changes.

Adding Peak Names Groups

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



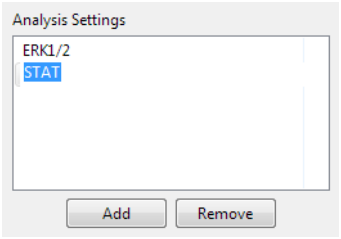
3. Click on the new group and enter a new name.



4. Enter information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 368.
5. Click **OK** to save changes.

Modifying a Peak Names Group

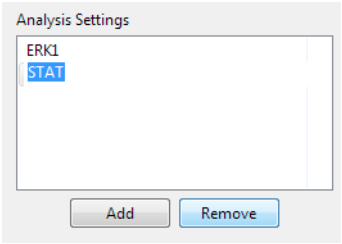
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Change the information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 368.
4. Click **OK** to save changes.

Deleting a Peak Names Group

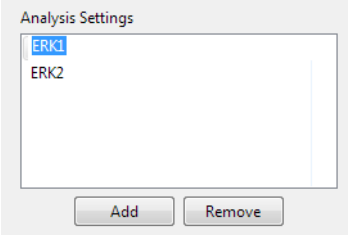
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



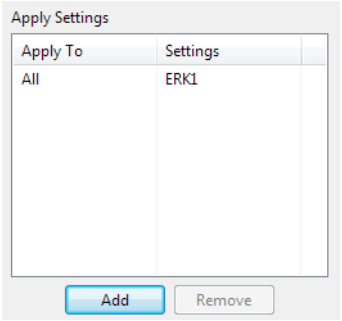
3. Click **OK** to save changes.

Applying Peak Names Groups to Run Data

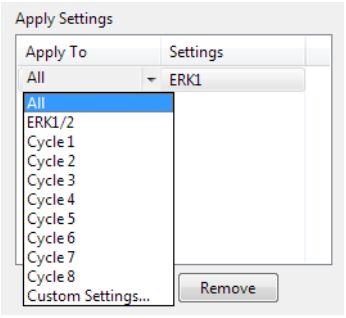
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.

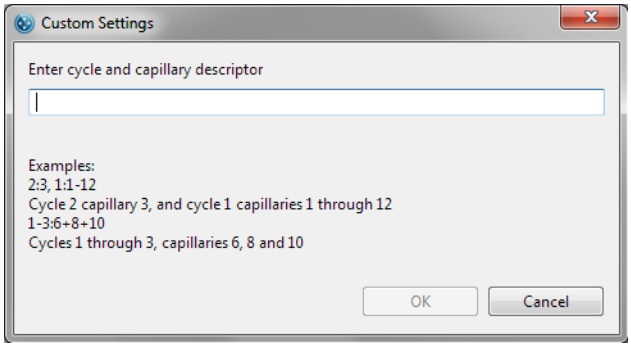


4. Click the cell in the **Apply To** column, then click the down arrow.

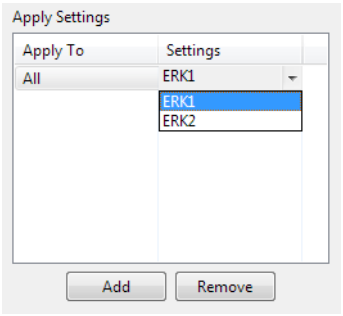


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **All** - When this option is selected, peak names group settings will be applied to all capillaries in the run file.
   - **Primary antibody names** - All primary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
   - **Attributes** - All primary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

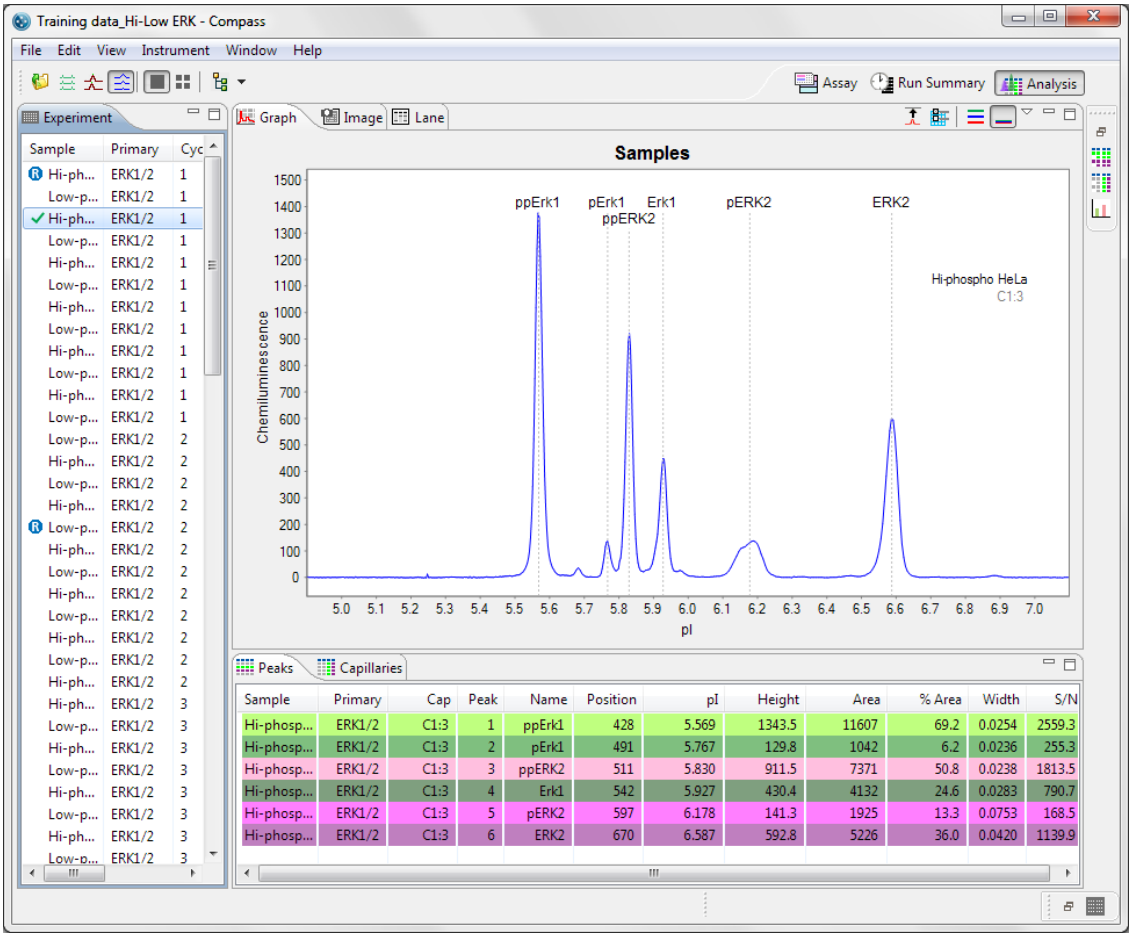


6. If you need to change the peak names group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.

9. Click **OK** to save changes. Named peaks will be identified with a peak name label in the electropherogram and virtual blot, and will be color coded in the peaks and capillaries tables:

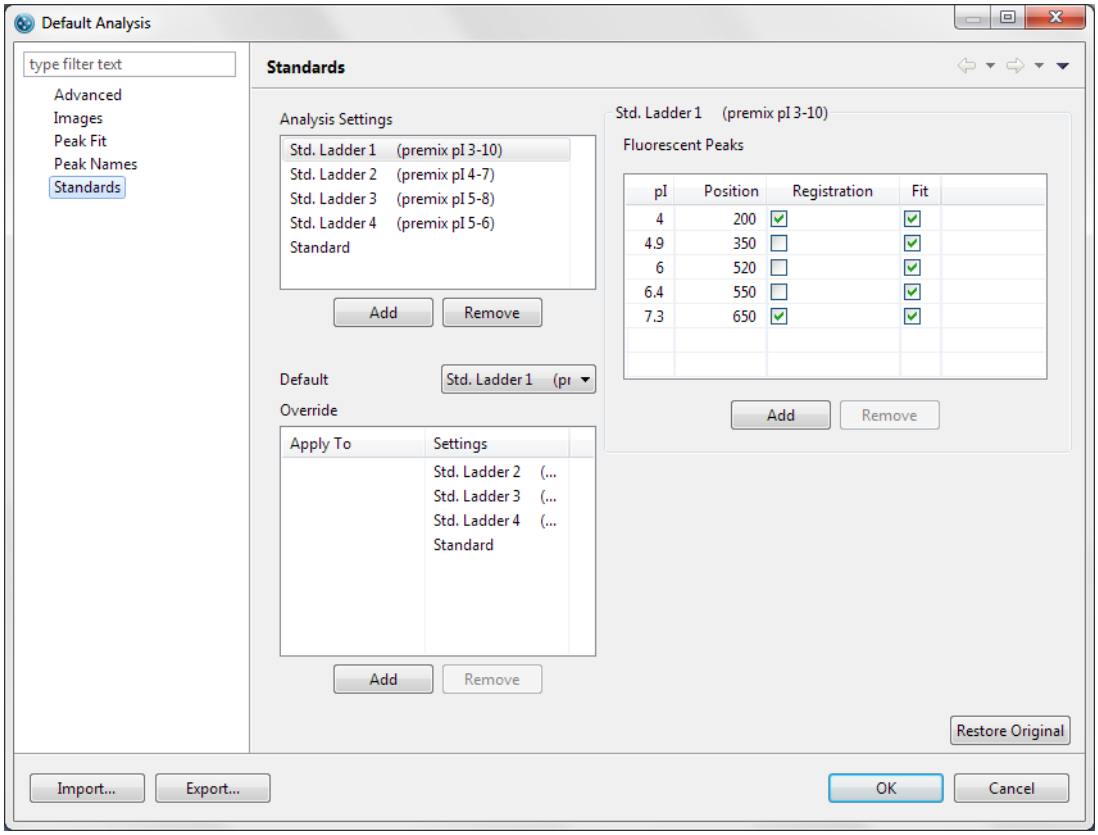


Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Hi-phosp...	ERK1/2	C1:3	1	ppErk1	428	5.569	1343.5	11607	69.2	0.0254	2559.3
Hi-phosp...	ERK1/2	C1:3	2	pErk1	491	5.767	129.8	1042	6.2	0.0236	255.3
Hi-phosp...	ERK1/2	C1:3	3	ppERK2	511	5.830	911.5	7371	50.8	0.0238	1813.5
Hi-phosp...	ERK1/2	C1:3	4	Erk1	542	5.927	430.4	4132	24.6	0.0283	790.7
Hi-phosp...	ERK1/2	C1:3	5	pERK2	597	6.178	141.3	1925	13.3	0.0753	168.5
Hi-phosp...	ERK1/2	C1:3	6	ERK2	670	6.587	592.8	5226	36.0	0.0420	1139.9

Standards Settings

The standards analysis settings page lets you view and change the pI and position for fluorescent standards and set the registration peaks. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 386.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 387.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.

- Click **Cancel** to exit without saving changes.

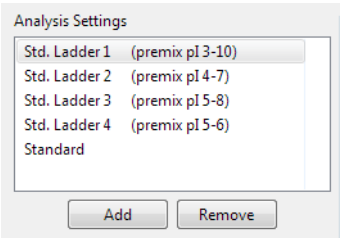
Standards Analysis Settings Groups

Standards settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
ProteinSimple recommends using the Compass default values for standards analysis settings. These settings are included in the default Standards group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 386.

Standards groups are displayed in the analysis settings box:

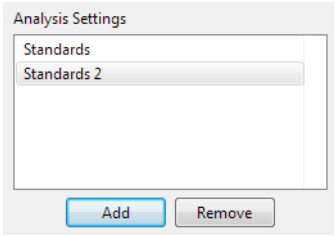


The Std. Ladder groups shown contains the Compass default analysis settings for pI Standard Ladders used with each of the premixes (separation gradients) for charge assays on Peggy. Users can select and use one of these default groups, make changes to groups or create new groups.

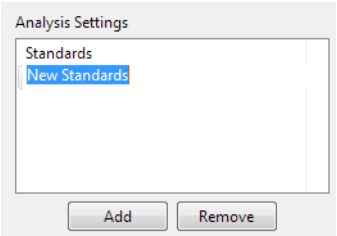
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Standards Group

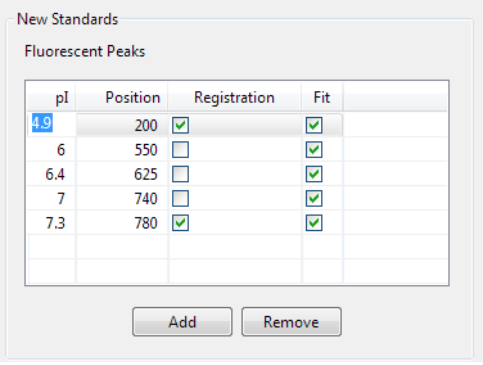
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



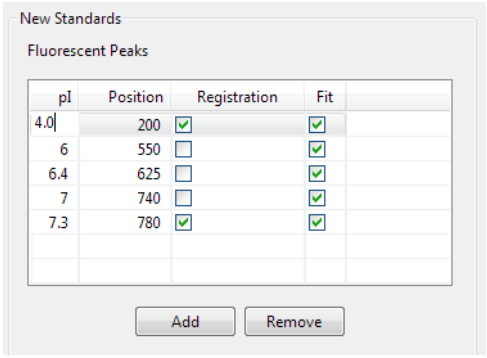
3. Click on the new group and enter a new name.



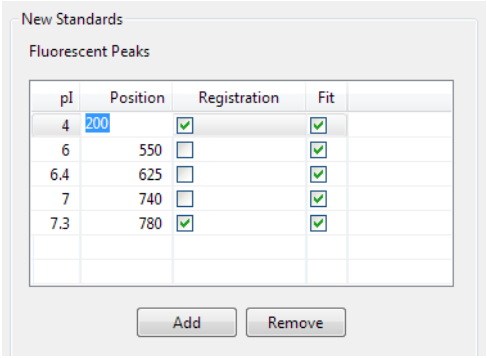
4. Click in the first cell in the **pI** column in the Fluorescent Peaks table.



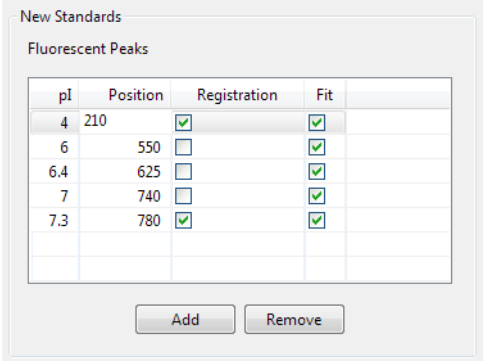
5. Enter the pI for the fluorescent standard.



6. Click in the first cell in the **Position** column.

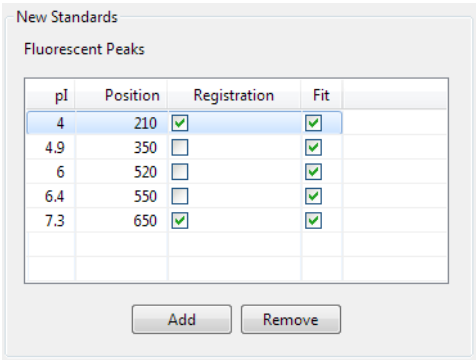


7. Enter the position of the fluorescent standard peak.



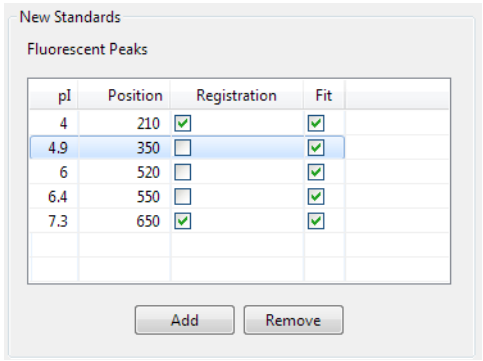
NOTE: Standards peak positions are relative to each other. Only the difference in their position is used to help identify the standard peaks. When entering standard peak information for the first time, review the standards data in the Analysis screen to find the correct peak position.

8. Repeat the steps above for the remaining standards in the table.
   - **To add another standard** - Click **Add** under the peak table, then modify the information in the new row.
   - **To remove a standard** - Select its row and click **Remove**.
9. Select which standard should be used for capillary registration by clicking the checkbox in the **Registration** column. The first and last standards are typically used for the registration.

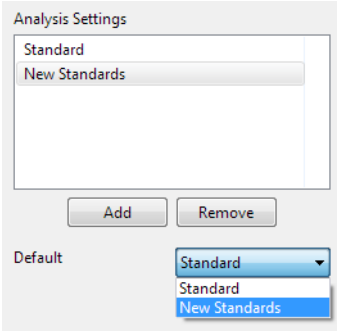


NOTE: In order for Compass to perform data analysis, at least one peak must be selected for registration.

10. Select which standards should be used for pI determination of sample proteins by clicking the checkbox in the **Fit** column. The standards not used for registration are typically also used for fit.



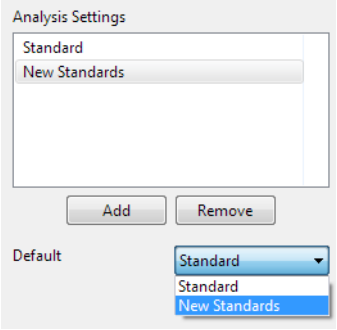
11. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.



12. Click **OK** to save changes.

Changing the Default Standards Group

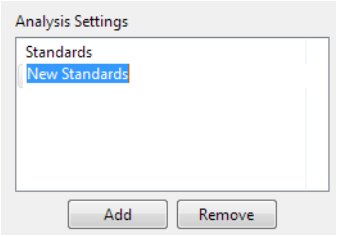
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying a Standards Group

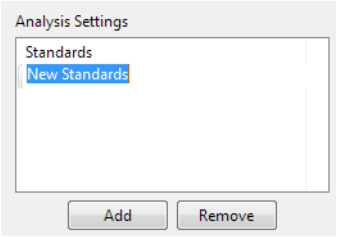
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify fluorescent standards information as described in "Creating a New Standards Group" on page 380.
4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

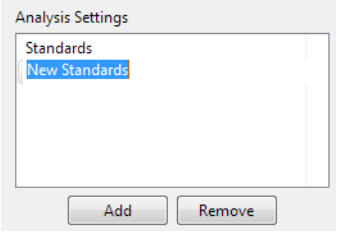
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



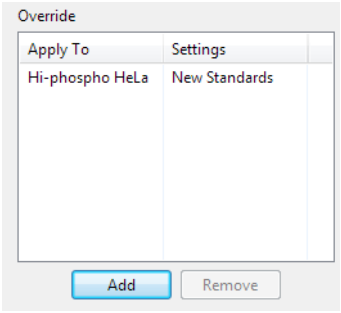
3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

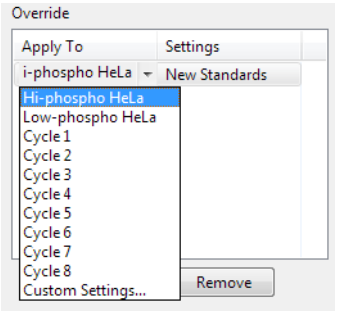
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

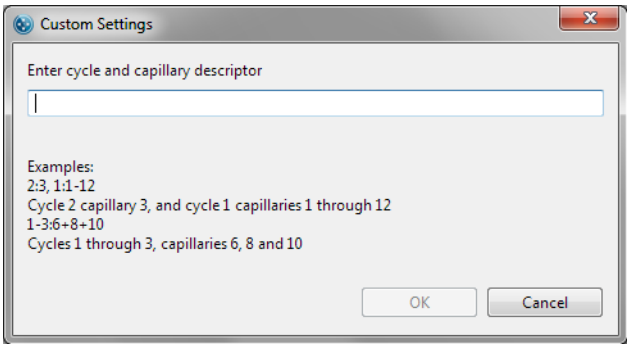


4. Click the cell in the **Apply To** column, then click the down arrow.

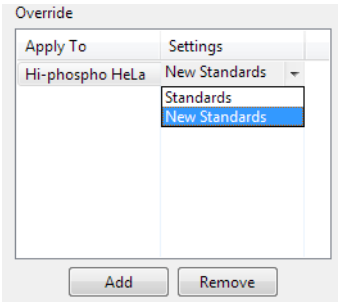


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Importing and Exporting Analysis Settings

The analysis settings in a run file can be exported as a separate file. This allows the same analysis settings to be imported into other assays or run files at a later time, rather than having to re-enter settings manually.

Importing Analysis Settings

NOTE: Importing an analysis settings file populates the settings in all Analysis pages.

---

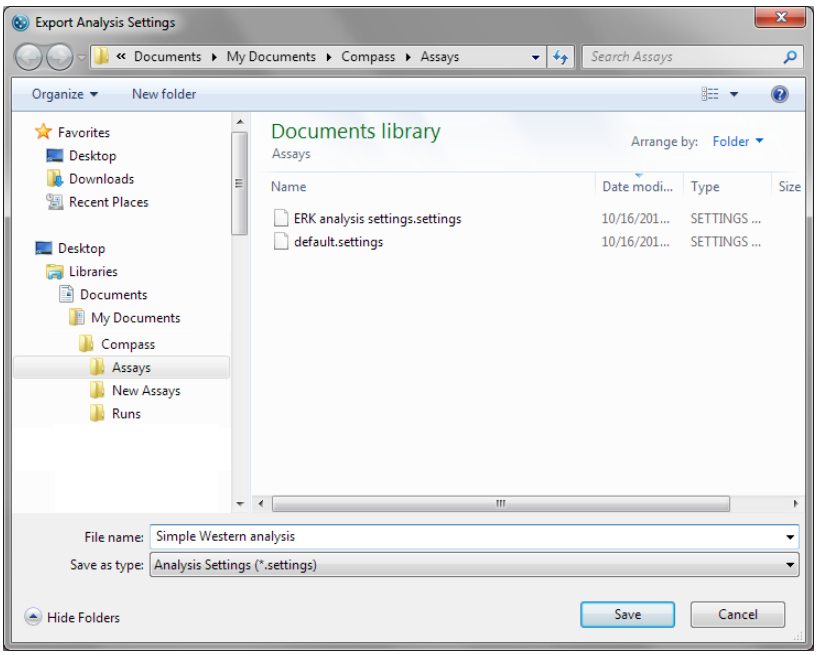
1. Open the run file or assay you want to import analysis settings to.

2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).
3. Click **Import** on any page.
4. Select a settings file (*.settings) and click **OK**. The imported settings will display in all analysis pages.

Exporting Analysis Settings

NOTE: Exporting an analysis settings file exports the settings in all Analysis pages.

1. Open the run file or assay you want to export analysis settings from.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).
3. Click **Export** on any page. The following window displays:



4. The default directory is Compass/Assays. Change the directory if needed.
5. Enter a file name and click **Save**. The settings will be saved as a *.settings file.

Chapter 10:

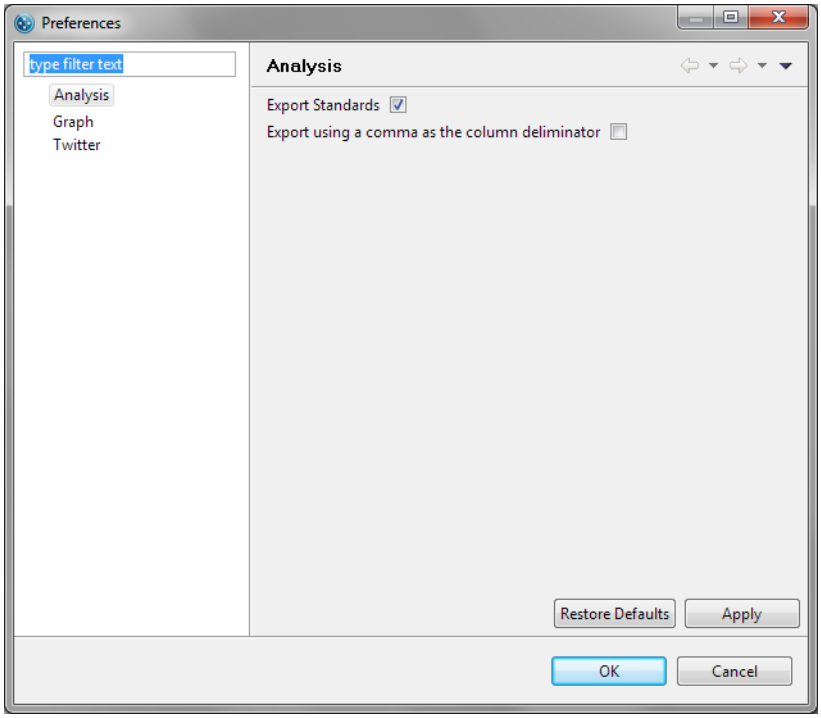
Setting Custom User Preferences

Chapter Overview

- Custom Preference Options
- Setting Data Export Options
- Selecting Custom Plot Colors for Graph Overlay
- Setting Peggy Up to Send Tweets

Custom Preference Options

Users can set and save custom preferences for data export, plot colors in the graph and Twitter communication. To access these settings, select **Edit** in the main menu and click **Preferences**.

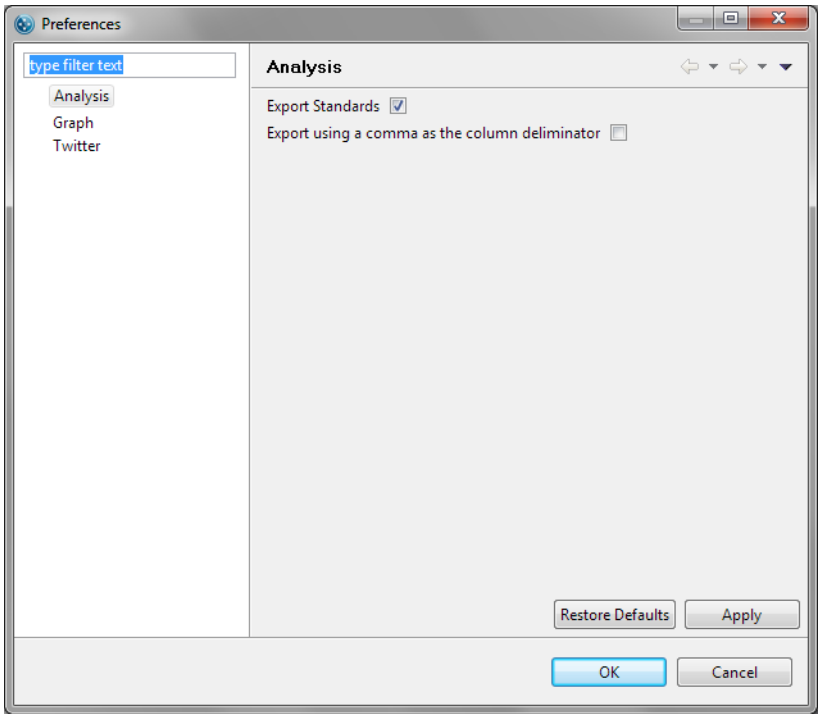


To move between preferences pages in this window, click on any option in the list on the left. The following items can be user-customized in Compass:

- **Analysis** - Lets you customize data export options.
- **Graph** - Lets you customize graph color displays.
- **Twitter** - Lets you configure Peggy to Tweet run status.

Setting Data Export Options

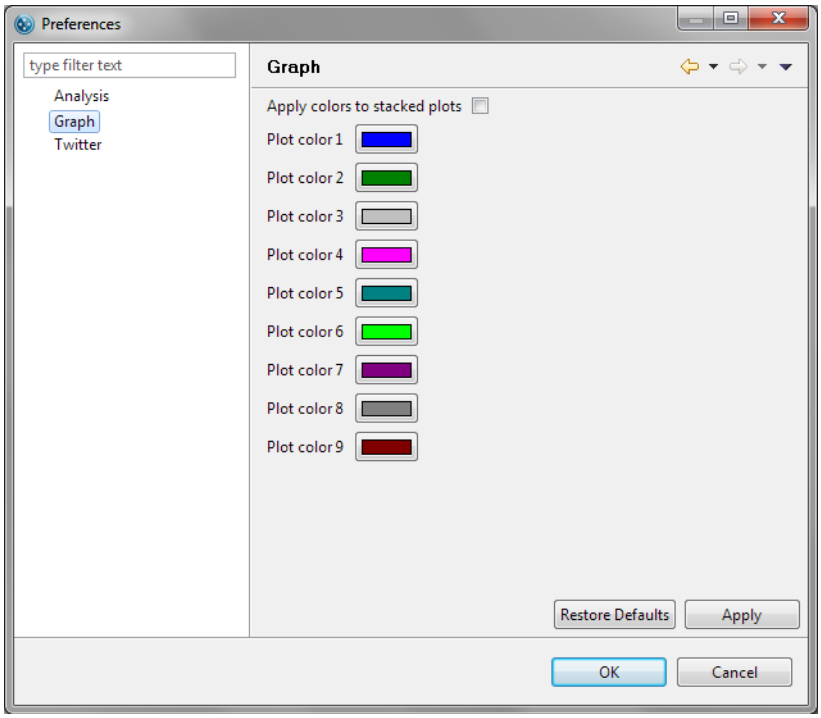
Select **Edit** in the main menu and click **Preferences**, then click **Analysis** in the options list.



- **Export Standards** - Selecting this option includes data for the standards in each sample when run data is exported. When this option is deselected, only sample data will be exported. This option is selected by default.
- **Export using a comma as the column delimiter -** Selecting this option exports run data in .csv format. When this option is deselected, the data is exported in .txt format.
- Click **Apply** to apply changes to any open run files in Compass.
- Click **Restore Defaults** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Selecting Custom Plot Colors for Graph Overlay

Select **Edit** in the main menu and click **Preferences**, then click **Graph** in the options list.



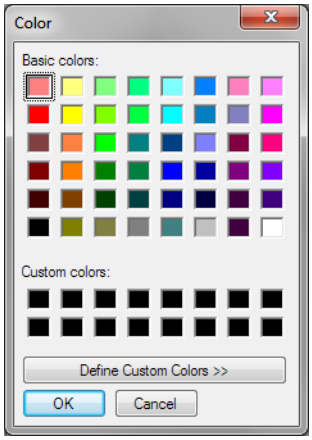
- **Apply colors to stacked plots** - Selecting this option applies the color scheme to individual electropherograms when the Stack the Plots option is selected in the Analysis screen graph pane.

NOTE: If Apply colors to stack plots is not checked, the colors shown in the preferences screen will be applied only to overlaid electropherograms when the Overlay the Plot option is selected in the graph pane.

- When this option is deselected, plots will use Compass default colors.
- Click **Apply** to apply changes to any open run files in Compass.
- Click **Restore Defaults** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Changing Plot Colors

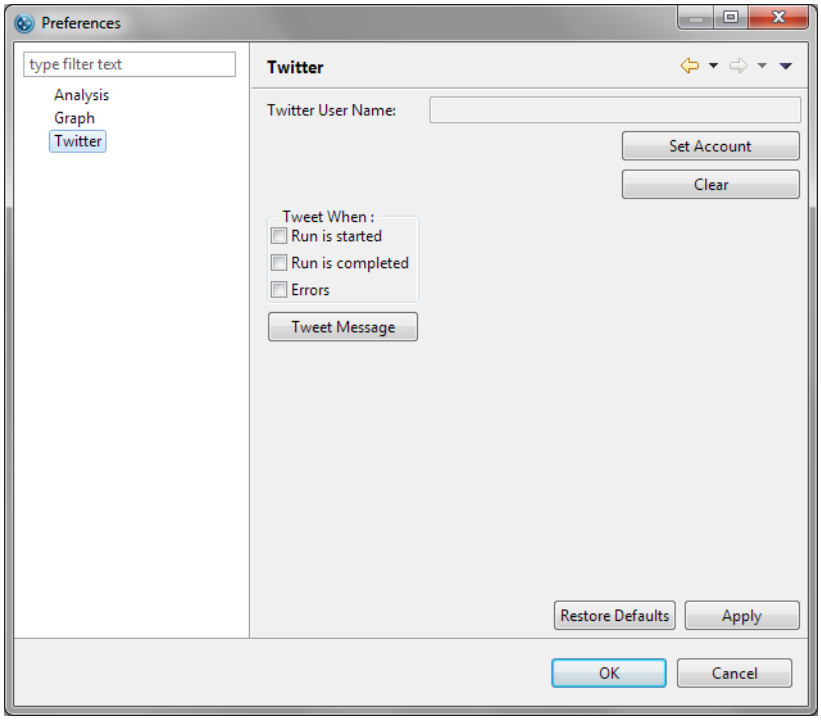
1. Select **Edit** in the main menu and click **Preferences**, then click **Graph** in the preferences list.
2. Click the color button next to a plot number. The color selection box displays:



3. Select a color or define a custom color and click **OK**. The color button will update to the new color selected.
4. Repeat the steps above for any other plot colors.
5. Check **Apply Colors to Stacked Plots** if you want the new color scheme to also be used for the Stack the Plots option in the graph pane.
6. Click **Apply** to apply changes to plots currently displayed in the graph pane.
7. Click **OK** to save changes and exit. When the Overlay the Plots option is selected in the graph pane, the new color scheme will be used.

Setting Peggy Up to Send Tweets

Select **Edit** in the main menu and click **Preferences**, then click **Twitter** in the options list.



- Click **Apply** to apply changes.
- Click **Restore Defaults** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

To have Peggy tweet a Twitter account:

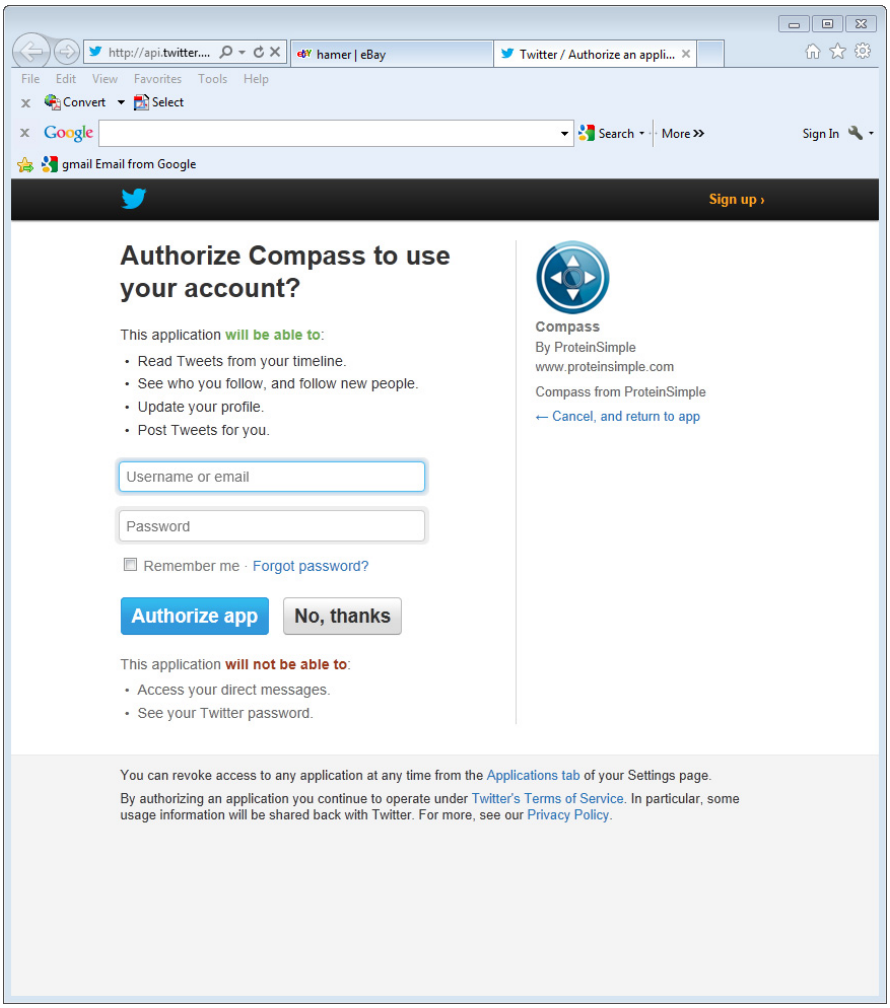
NOTES:
To set Peggy up to tweet, the computer you are using must have an internet connection.

To tweet, Peggy must be connected to the internet through a network connection or via the local lab computer.

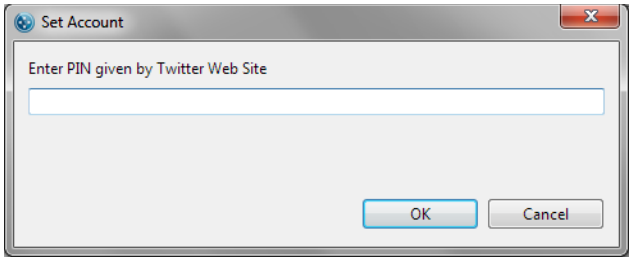
ProteinSimple recommends setting up a separate Twitter account for Peggy. This lets multiple people in the lab follow run progress. It also lets users send tweets directly from Peggy to all users, for example to notify others when the instrument is available or when an error has been reset, etc.

---

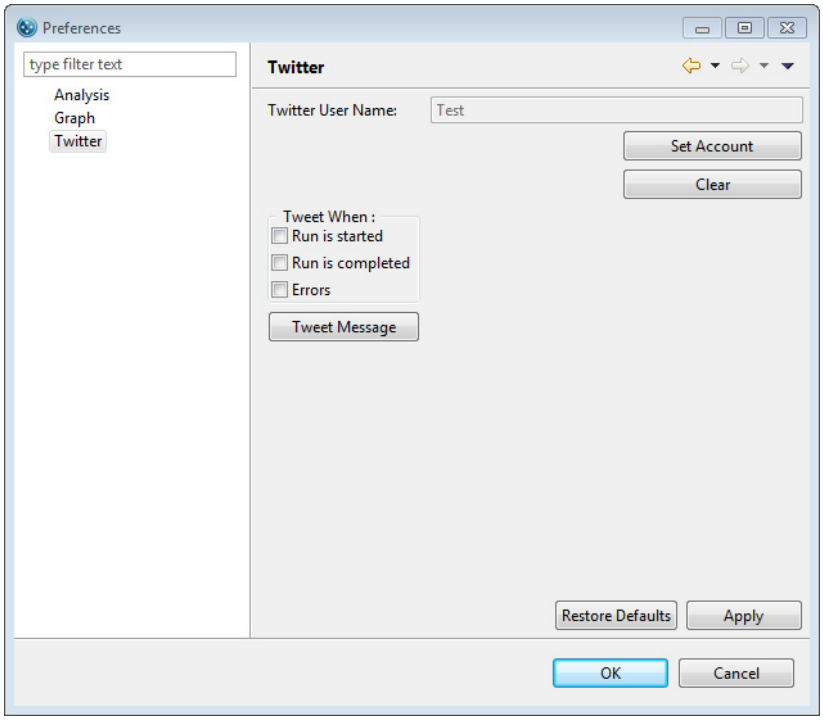
1. Click **Set Account**. A set account window will display in Compass and the following browser window will open:


Authorize Compass to use your account?
This application will be able to:
• Read Tweets from your timeline.
• See who you follow, and follow new people.
• Update your profile.
• Post Tweets for you.
Username or email
Password
Remember me · Forgot password?
Authorize app
No, thanks
This application will not be able to:
• Access your direct messages.
• See your Twitter password.
Compass
By ProteinSimple
www.proteinsimple.com
Compass from ProteinSimple
← Cancel, and return to app
You can revoke access to any application at any time from the Applications tab of your Settings page.
By authorizing an application you continue to operate under Twitter's Terms of Service. In particular, some usage information will be shared back with Twitter. For more, see our Privacy Policy.

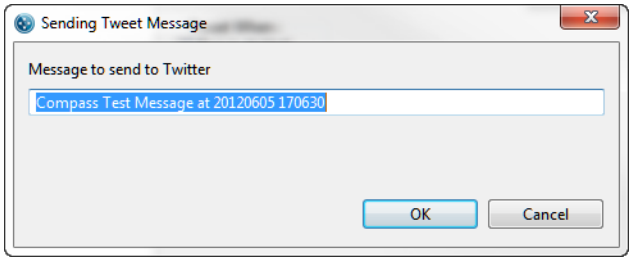
2. Enter a user name or email and password, then click **Authorize app**. A new page will display in the browser with a PIN number.
3. Enter the PIN number in the set account window in Compass and click **OK**:



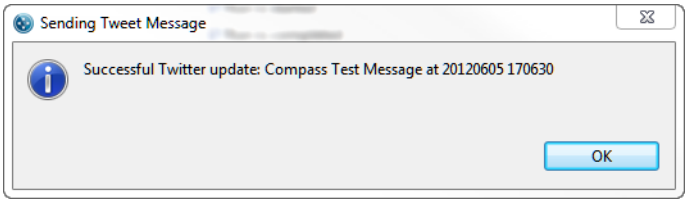
4. The user name will now appear in the Twitter User Name box. Select one or all of the Peggy tweet options in the Tweet When box, then click **Apply**.



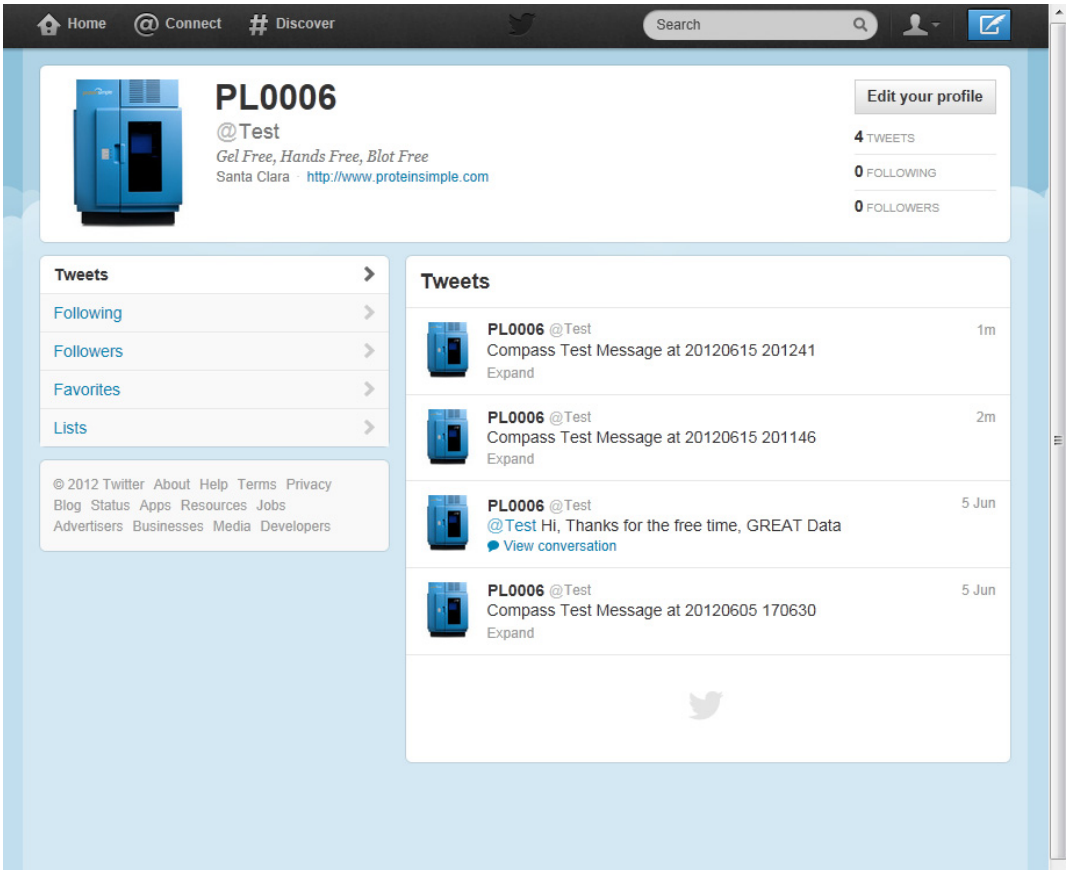
5. To confirm the Twitter account is receiving messages, click **Tweet Message**. Enter a test message and click **OK**.



6. If the test Tweet was successful, Compass will display the following message:



7. Click **OK** to save changes and exit. Peggy will automatically tweet as the selected options occur, as shown below:



Changing the Twitter Account

To change the Twitter account Peggy uses:

1. Select **Edit** in the main menu and click **Preferences**, and click **Twitter** in the preferences list.
2. Click **Clear**.
3. Set up the new account as described in "Setting Peggy Up to Send Tweets" on page 394.

Sending Manual Tweets from Peggy

You can send tweets directly from Peggy. For example, you may want to notify other users that the instrument is available, being serviced or when an error has been cleared. To do this:

1. Select **Edit** in the main menu and click **Preferences** and click **Twitter** in the preferences list.
2. Click **Tweet Message**.
3. Enter a test message and click **OK**. The tweet will be received by any users following the Twitter account Peggy uses.

Chapter 11:

Compass Access Control and 21 CFR Part 11 Compliance

Chapter Overview

- Overview
- Enabling Access Control
- Logging In to Compass
- Saving Changes
- Signing Files
- Instrument Command Log
- Run File History
- Troubleshooting Problems and Suggested Solutions
- Authorization Server

Overview

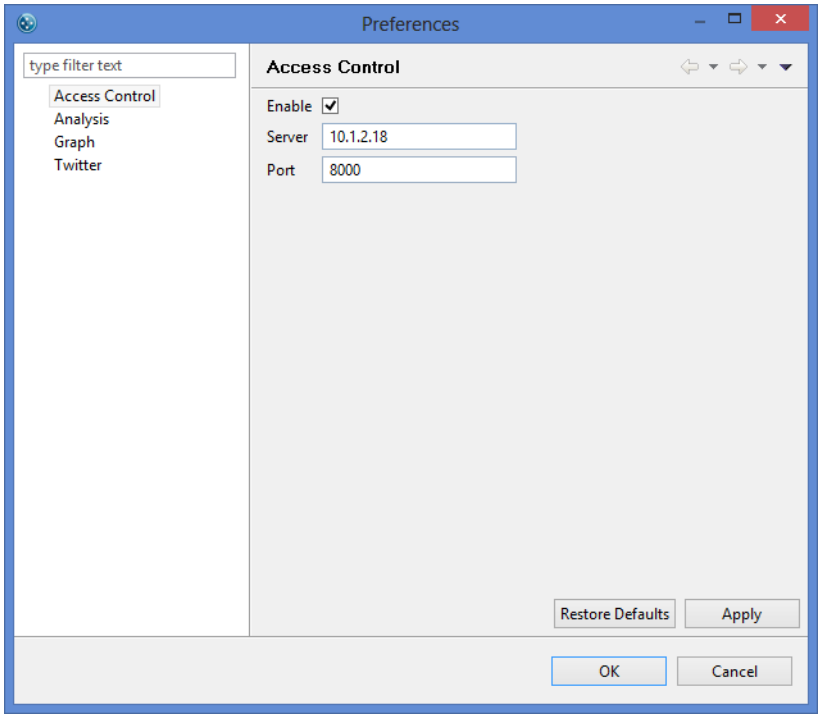
The Compass Access Control feature can be used to help satisfy the 21CFR Part 11 data security requirements when using Simple Western instruments. When Access Control is enabled and the Authorization Server has been installed (see "Authorization Server" on page 410):

- Users are required to log in to Compass when the software is launched
- A history of all actions is maintained
- Data files are signed and encrypted to prevent unauthorized changes (*e.g.*, all files are controlled)
- Each instrument maintains a history of user commands
- Each assay and data file includes a history of signed changes to the file

Compass can be run with or without Access Control enabled. When Access Control is disabled, no user log in is required and files are not encrypted or signed. The instrument history and file history are still maintained but the entries are not signed.

Enabling Access Control

Access Control is enabled in **Preferences**. Select **Edit** in the main menu, click **Preferences**, then select **Access Control**.



To enable Access Control:

1. Check the **Enable** box.
2. Enter the IP address of the Authorization server. Use format X.X.X.X or LocalHost if installing the server on the local machine.

NOTE: Always use the default port setting of 8000, this should not be changed.

---

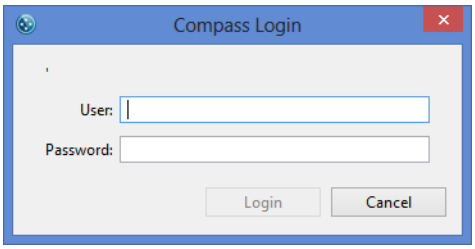
3. Close Compass. The next time Compass is launched, a user log in will be required.

---

*NOTE: Access Control can only be disabled by logging into Compass and deselecting the **Enable** box in the Access Control page of Preferences.*

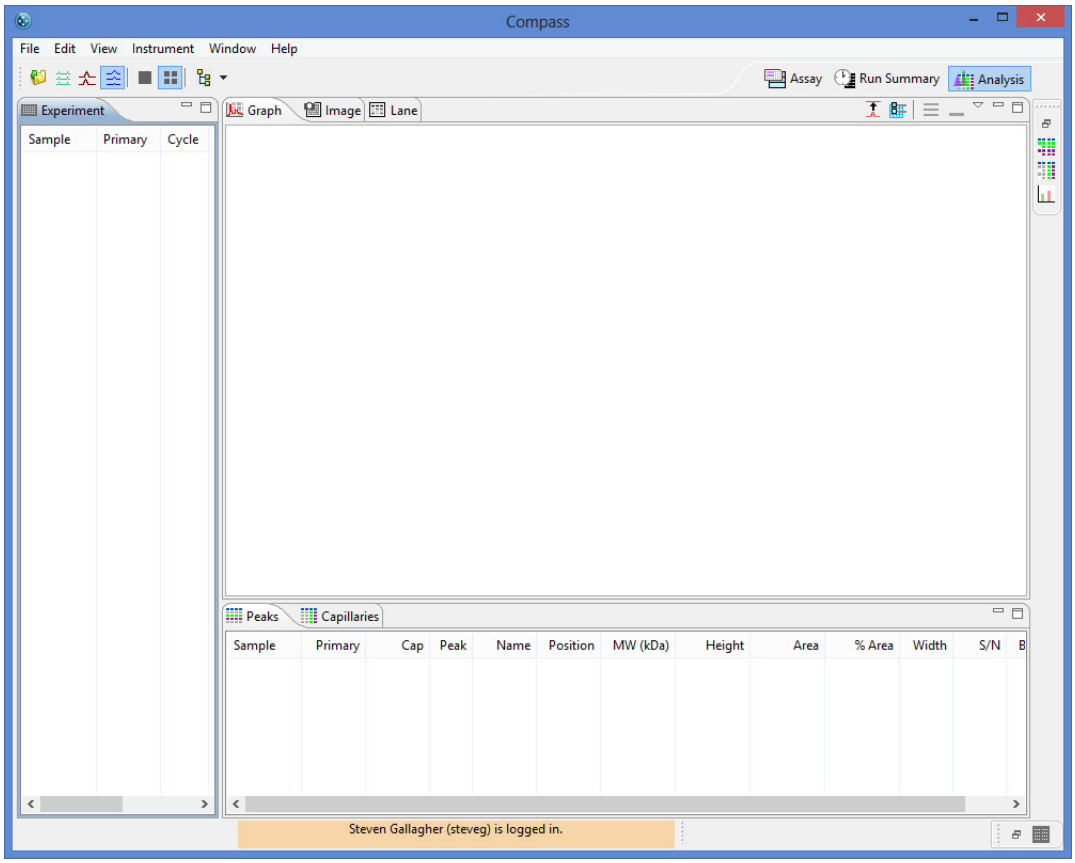
Logging In to Compass

With Access Control enabled, all users must log in to Compass whenever the software is launched.

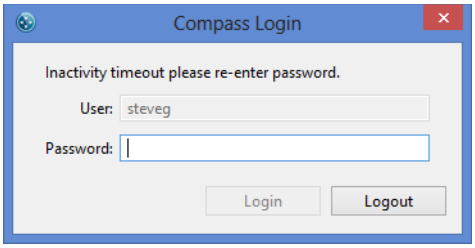


Enter your user name and password previously setup by your Compass Administrator.

A successful log in will display the Compass main window with the user information in the lower status bar. The full user name is displayed with the unique user ID in parenthesis:



If there is no activity in Compass for 20 minutes, the user must re-enter their password to perform any controlled actions:

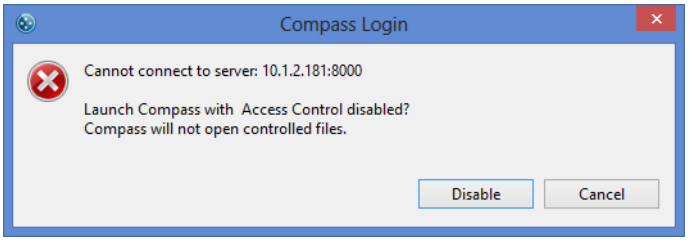


Resolving Log In Issues

Log in failures may occur when:

- The server is temporarily unavailable
- Compass is using the wrong IP address

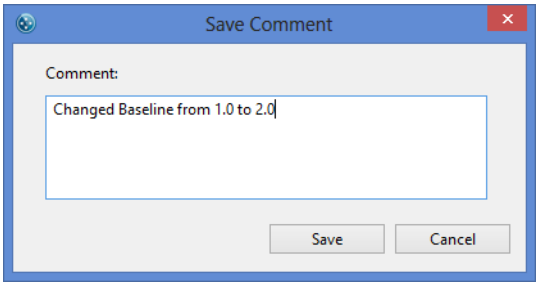
When this happens, the following message displays:



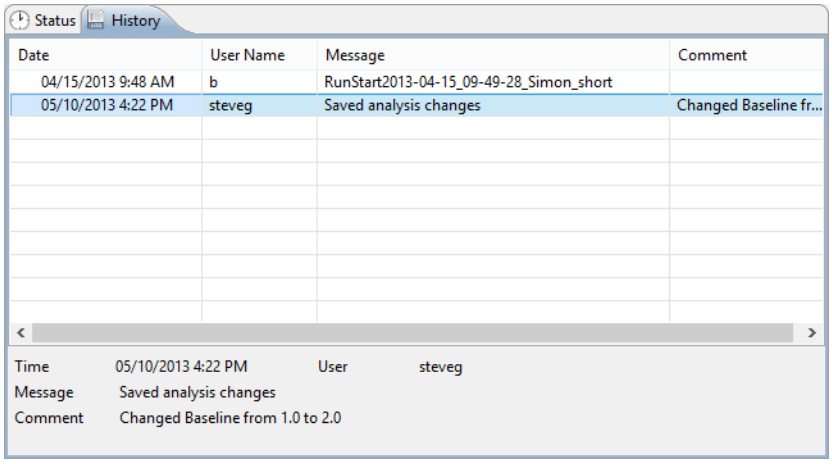
Click **Disable** to restart Compass with Access Control disabled. Verify or correct the server IP address then close and restart Compass to log in with Access Control enabled.

Saving Changes

When **Save** is selected from the **File** menu, a dialog box will display to allow you to enter a comment before saving the signed file:

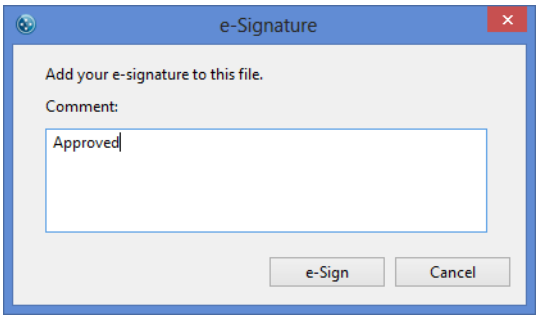


The comment is added to the signature entry in the file History:

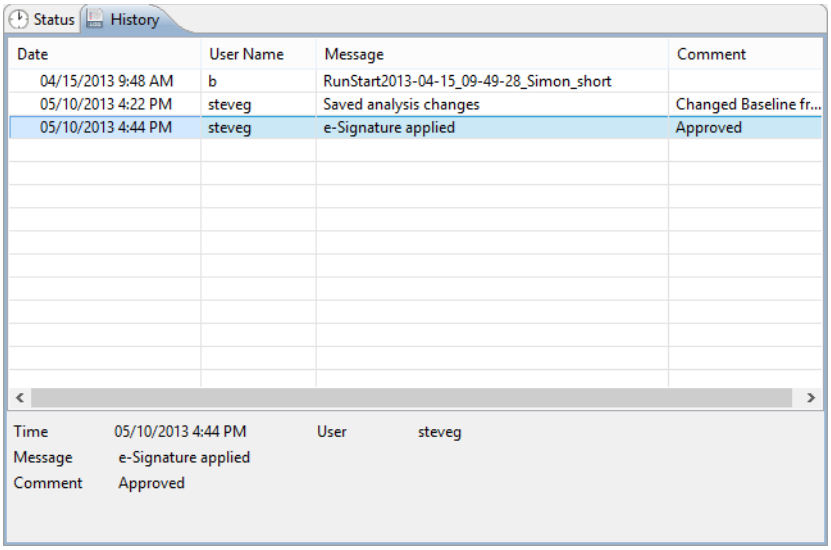


Signing Files

Select **e-Signature** from the **File** menu to add an electronic signature to a file.

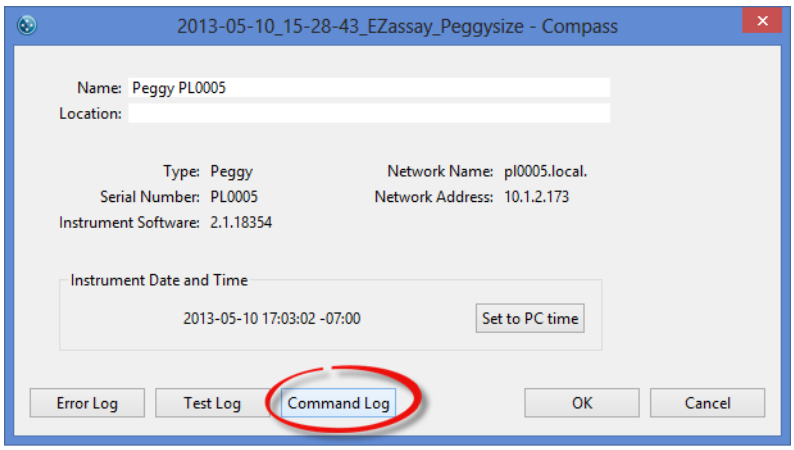


The signed entry will be added to the file History with the meaning of the signature entered in the comment, such as *Approved* or *Verified*.

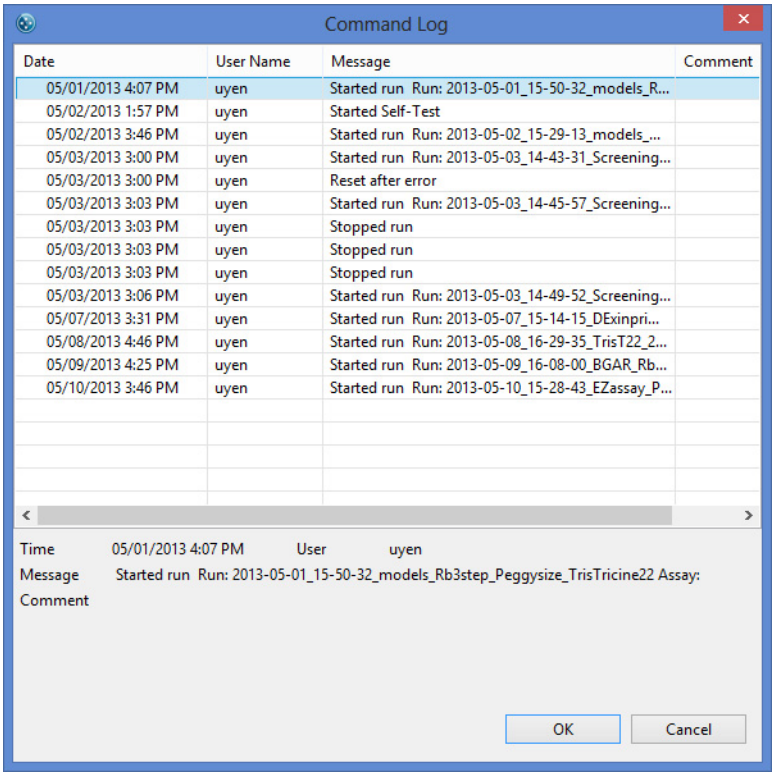


Instrument Command Log

The Instrument Command Log can be viewed at any time by selecting the **Instrument** menu and clicking **Properties**, and then clicking the **Command Log** button:

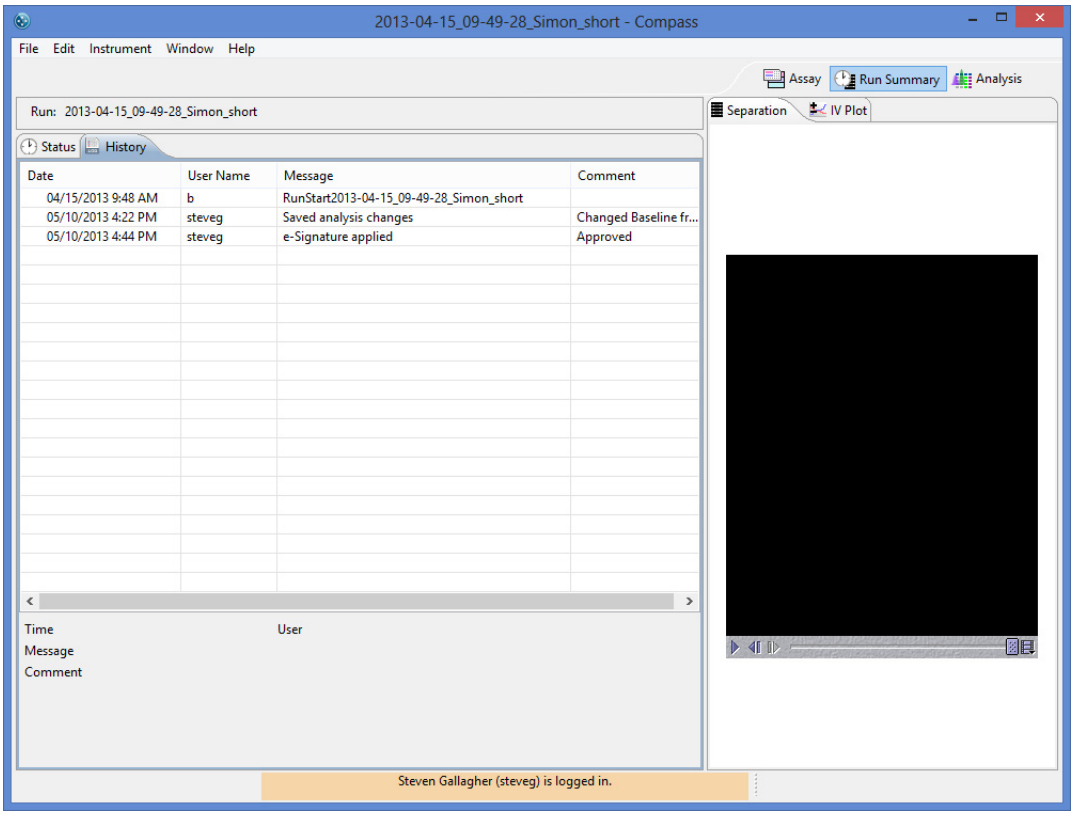


The Command Log lists all the commands sent to the instrument that were signed by the user who sent the command. If you want to copy the Command Log at any time, right click in the table and select **Copy**, then paste into another document.


Command Log
Date
User Name
Message
Comment
05/01/2013 4:07 PM uyen Started run Run: 2013-05-01_15-50-32_models_R...
05/02/2013 1:57 PM uyen Started Self-Test
05/02/2013 3:46 PM uyen Started run Run: 2013-05-02_15-29-13_models_...
05/03/2013 3:00 PM uyen Started run Run: 2013-05-03_14-43-31_Screening...
05/03/2013 3:00 PM uyen Reset after error
05/03/2013 3:03 PM uyen Started run Run: 2013-05-03_14-45-57_Screening...
05/03/2013 3:03 PM uyen Stopped run
05/03/2013 3:03 PM uyen Stopped run
05/03/2013 3:03 PM uyen Stopped run
05/03/2013 3:06 PM uyen Started run Run: 2013-05-03_14-49-52_Screening...
05/07/2013 3:31 PM uyen Started run Run: 2013-05-07_15-14-15_DExinpri...
05/08/2013 4:46 PM uyen Started run Run: 2013-05-08_16-29-35_TrisT22_2...
05/09/2013 4:25 PM uyen Started run Run: 2013-05-09_16-08-00_BGAR_Rb...
05/10/2013 3:46 PM uyen Started run Run: 2013-05-10_15-28-43_EZassay_P...
Time 05/01/2013 4:07 PM User uyen
Message Started run Run: 2013-05-01_15-50-32_models_Rb3step_Peggysize_TrisTricine22 Assay:
Comment
OK
Cancel

Run File History

Select the **Run Summary** screen tab and then the **History** tab to see the file History. To copy the file History, right click in the table and select **Copy**, then paste into another document.


2013-04-15_09-49-28_Simon_short - Compass
File Edit Instrument Window Help
Assay Run Summary Analysis
Run: 2013-04-15_09-49-28_Simon_short
Separation IV Plot
Status History
Date User Name Message Comment
04/15/2013 9:48 AM b RunStart2013-04-15_09-49-28_Simon_short
05/10/2013 4:22 PM steveg Saved analysis changes Changed Baseline fr...
05/10/2013 4:44 PM steveg e-Signature applied Approved
Time User
Message
Comment
Steven Gallagher (steveg) is logged in.

Troubleshooting Problems and Suggested Solutions

If any of the following error messages are encountered, follow the recommended steps below to resolve the issue.

- **Unknown user name or password**.
  - Check if the Caps Lock is on, user name and password are case sensitive.
  - Ask a Compass administrator to confirm your user name. If your password is unknown then the administrator can reset your password (see "Resetting User Passwords" on page 418 for more information).

- **Server not available**.
  - From the **Edit** menu, click **Preferences** and then **Access Control** to confirm the server address is set to the correct Authorization server address. Compass must be able to reach the server on the network.
  - The server must have inbound access to port 8000 enabled.
- **Controlled file cannot be opened without log in.** To open a controlled Run file, enable Access Control by clicking **Edit**, then **Preferences** and **Access Control.** Select **Enable**, close Compass, then re-launch Compass with a valid log in.
- **Uncontrolled file cannot be opened when logged in.** To open an uncontrolled Run file, disable Access Control by clicking **Edit**, then **Preferences** and **Access Control.** Deselect **Enable**, close Compass then re-launch the software.

NOTE: Uncontrolled Assay files can be opened when Compass Access Control is enabled (controlled mode).

- **Command disabled.** Certain commands are only available when a user with the correct permissions is logged in. To change user permissions, use a web browser to log in to the Authorization server web interface at the address shown on the **Access Control** page in **Preferences**, such as: 10.1.3.231:8000.
- **Compass does not prompt for log in.** Compass will only prompt for a log in on launch when Access Control is enabled in Preferences. Enable Access Control by clicking **Edit**, then **Preferences** and **Access Control.** Select **Enable**, close Compass, then re-launch the software. You should now be prompted for a log in.

Authorization Server

The Authorization Server controls the log in access to Compass. In the simplest configuration, the server is run on the same computer as Compass and only that copy of Compass is controlled. A single server can also be used to control access to multiple copies of Compass running on different computers, so long as they have network access to the server. Multiple copies of the server may be run on the same network, and each server will have its own user database.

To enable Compass to use a particular Authorization Server, click **Edit**, then **Preferences** and **Access Control** and enter the server IP address using format X.X.X.X.

NOTES:
Always use the default port setting of 8000, this should not be changed.

If the server is installed on the same computer as Compass (e.g., the local machine), enter LocalHost instead of the IP address. Contact your local IT Administrator to assist with installing the Authorization Server in your preferred format.

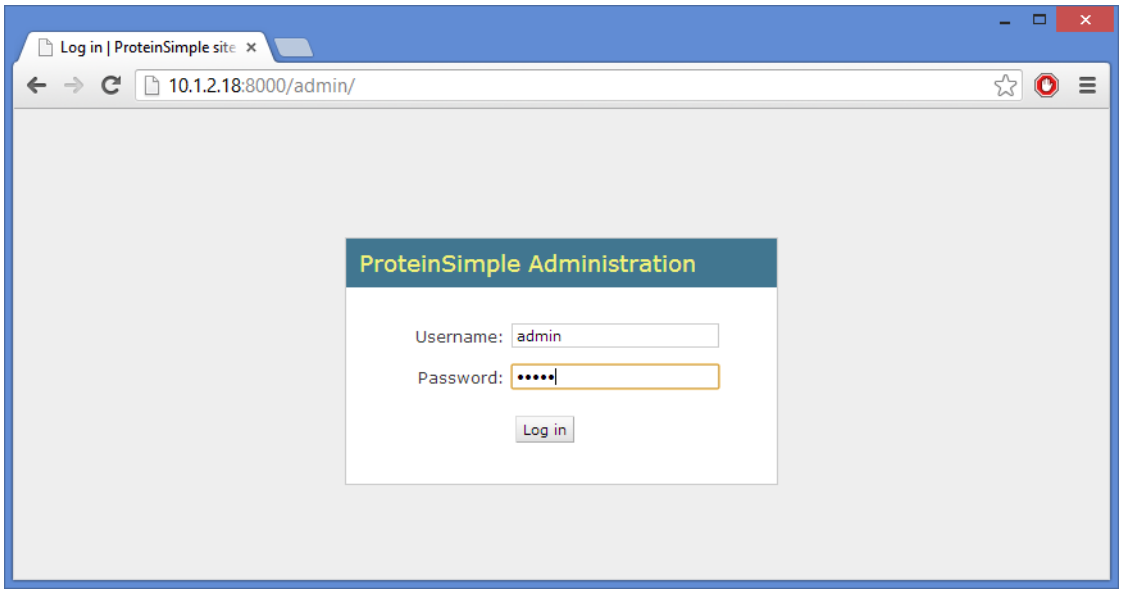
Server Administration

The Authorization Server is configured through a web interface at the IP address of the server on port 8000. To access the Server home page, open any browser and type the IP address on port 8000 in a X.X.X.X:8000 or http://X.X.X.X:8000 format. Use LocalHost instead of the IP address if the Server is installed on the local machine.

The default server administrator is:

- User: admin
- Password: admin

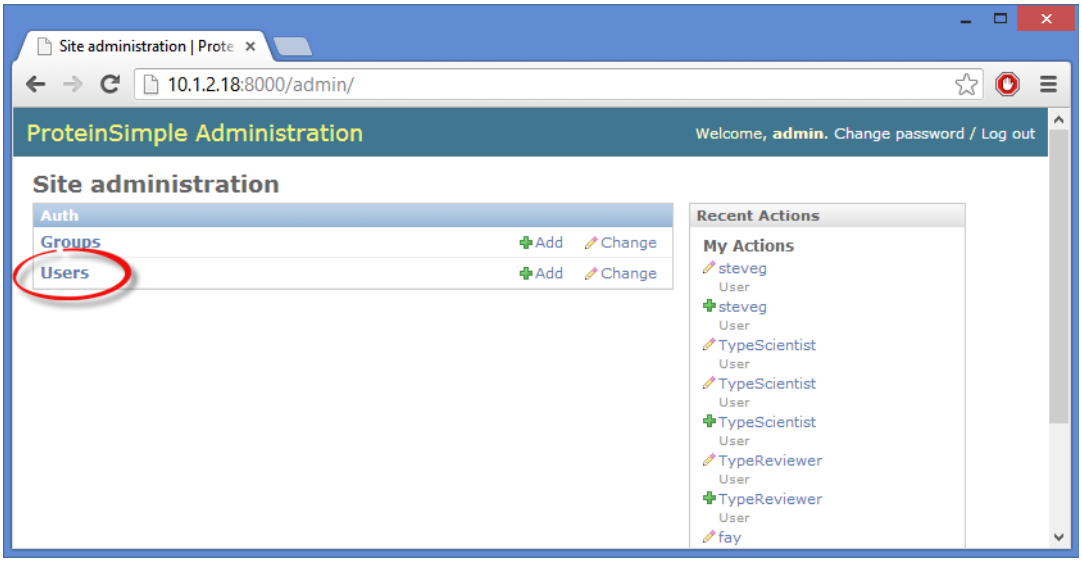
After installing the Authorization Server, the administrator user name and password can be changed.



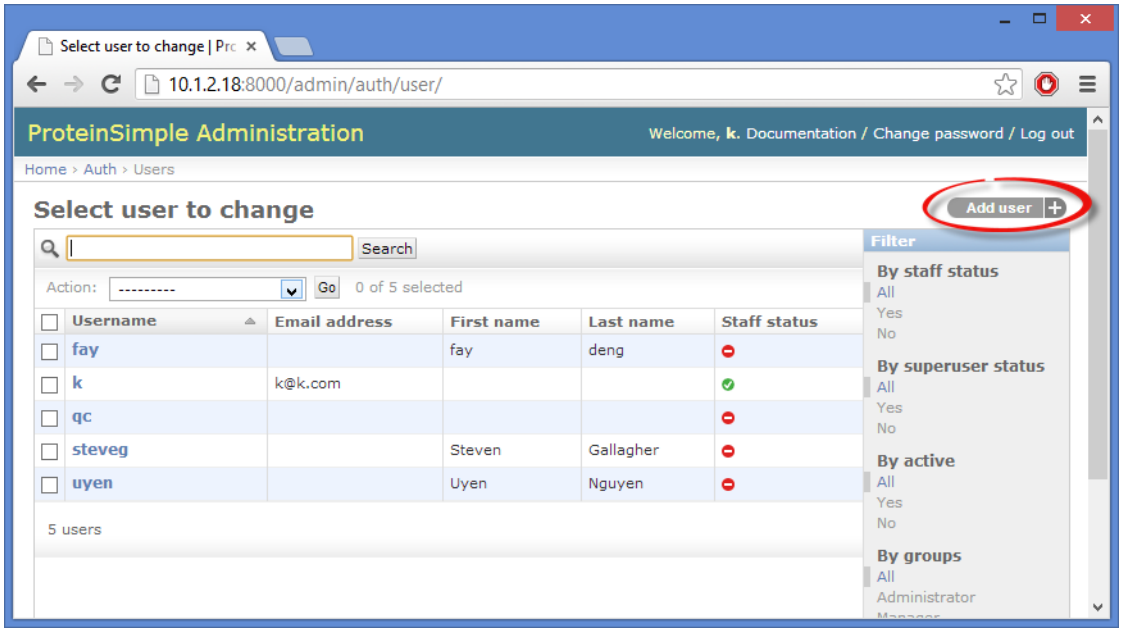
Adding Non-admin Users

Add a user to the server to allow that user to log in to Compass. To do this:

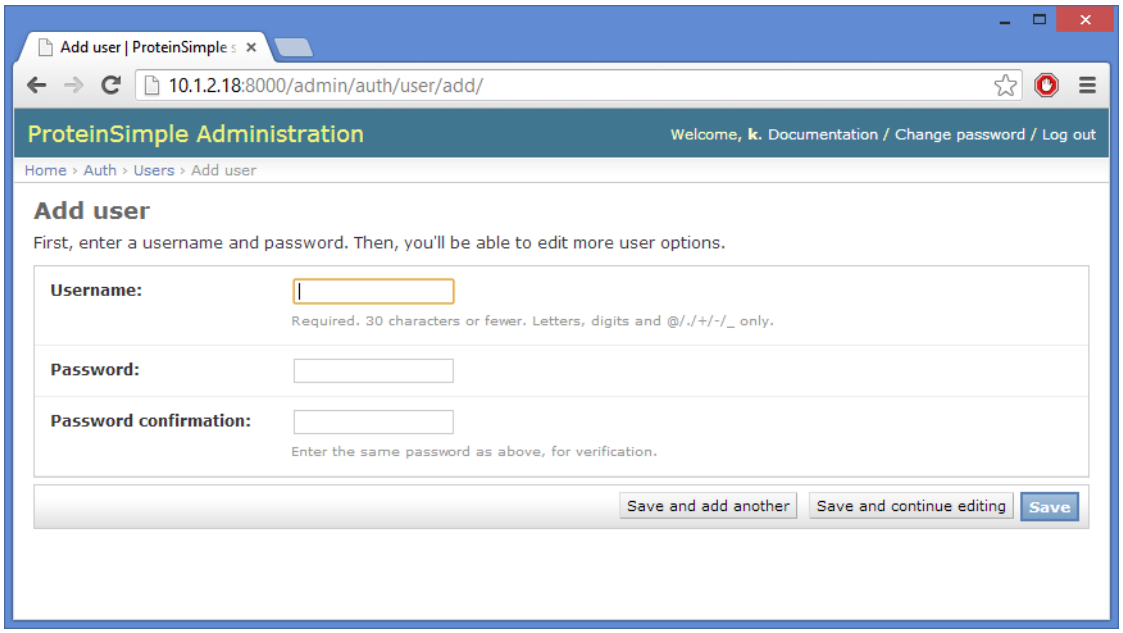
1. Select **Users** from the Site Administration home page:



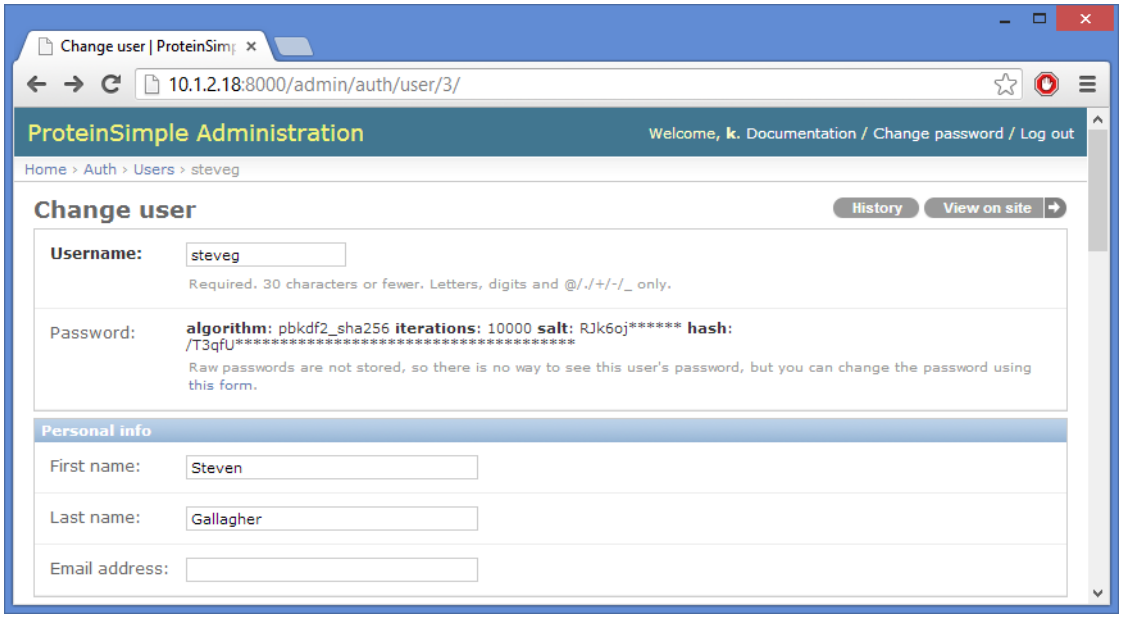
2. From the Users page, select **Add User**:



3. Fill in the fields to create a new user:


Add user | ProteinSimple
10.1.2.18:8000/admin/auth/user/add/
ProteinSimple Administration
Welcome, k. Documentation / Change password / Log out
Home › Auth › Users › Add user
Add user
First, enter a username and password. Then, you'll be able to edit more user options.
Username:
Required. 30 characters or fewer. Letters, digits and @/./+/-/_ only.
Password:
Password confirmation:
Enter the same password as above, for verification.
Save and add another
Save and continue editing
Save

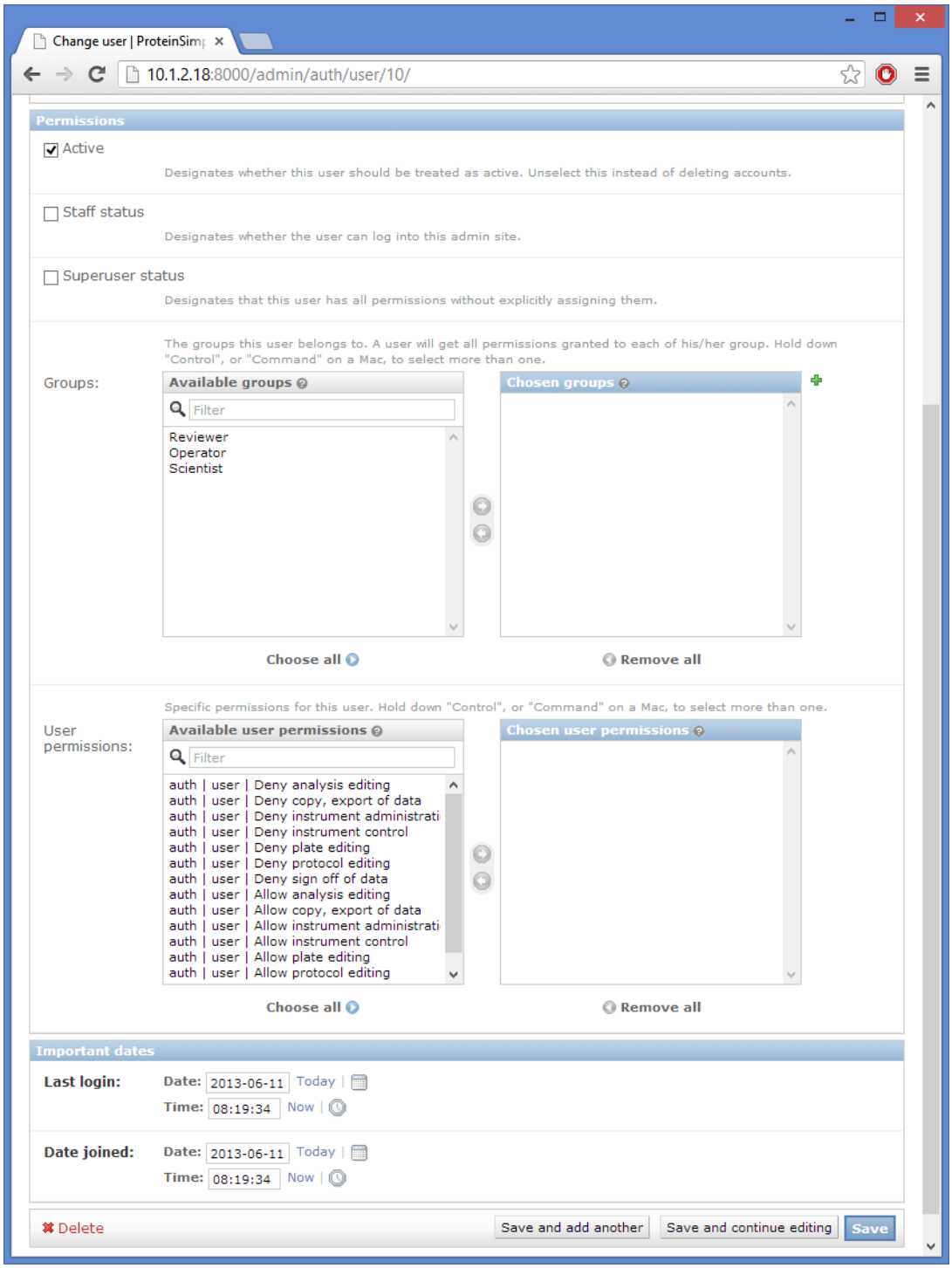
After adding a new user more information can be added:


Change user | ProteinSim
10.1.2.18:8000/admin/auth/user/3/
ProteinSimple Administration
Welcome, k. Documentation / Change password / Log out
Home › Auth › Users › steveg
Change user
History
View on site
Username:
steveg
Required. 30 characters or fewer. Letters, digits and @/./+/-/_ only.
Password:
algorithm: pbkdf2_sha256 iterations: 10000 salt: RJk6oj****** hash:
/T3qfU**********
Raw passwords are not stored, so there is no way to see this user's password, but you can change the password using this form.
Personal info
First name:
Steven
Last name:
Gallagher
Email address:

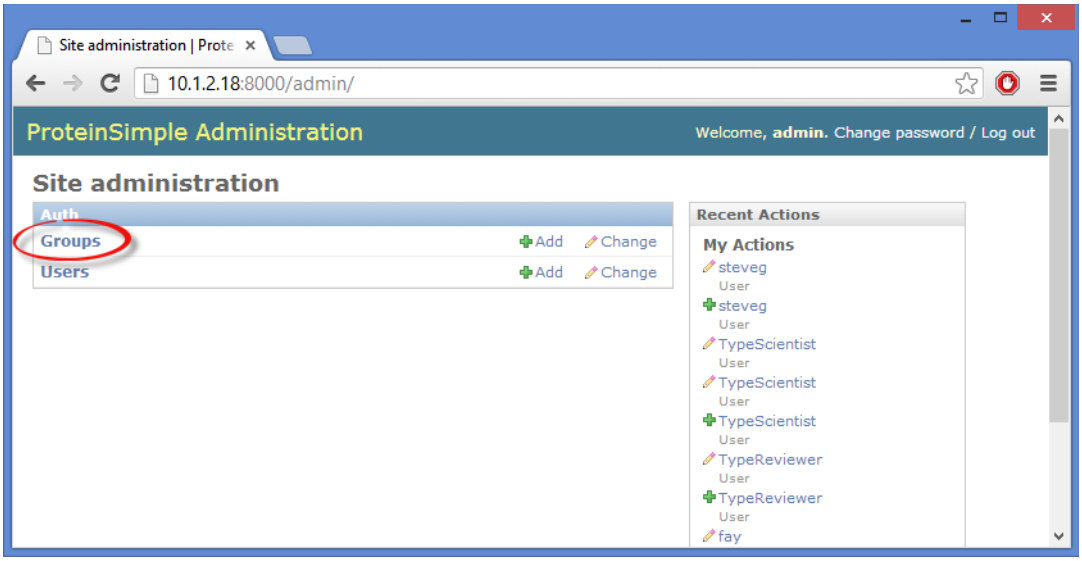
Permissions

All users can log in to Compass, but the commands available within Compass are controlled by Permission settings. Commands a user does not have permission to use will be disabled. After user permissions have been changed on the server the user must close and re-open Compass to use the new permissions.

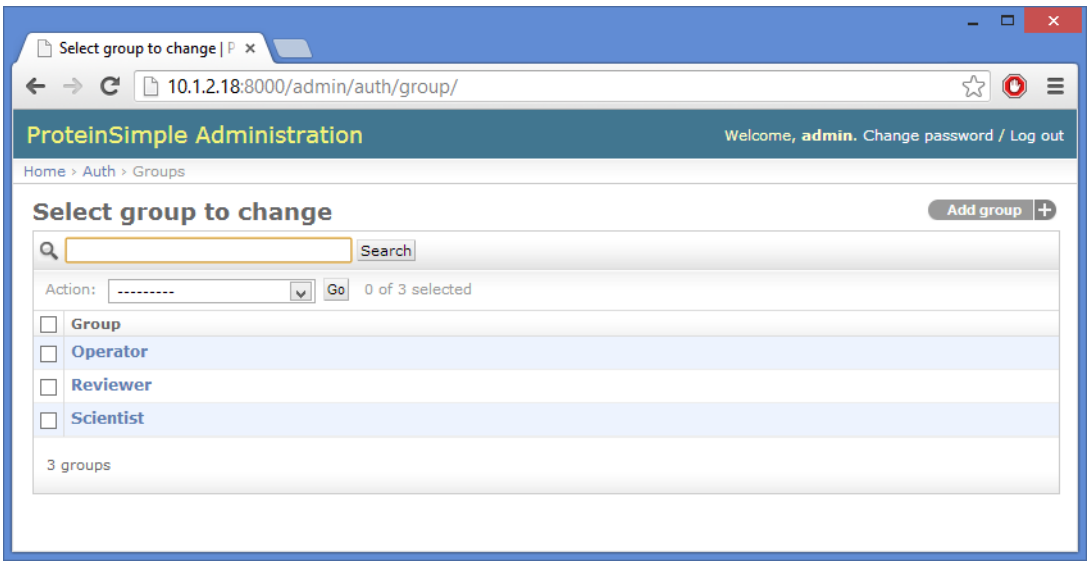
Users can belong to groups that have multiple permissions such as Manager or Scientist:


Change user | ProteinSim
10.1.2.18:8000/admin/auth/user/10/
Permissions
Active
Designates whether this user should be treated as active. Unselect this instead of deleting accounts.
Staff status
Designates whether the user can log into this admin site.
Superuser status
Designates that this user has all permissions without explicitly assigning them.
The groups this user belongs to. A user will get all permissions granted to each of his/her group. Hold down "Control", or "Command" on a Mac, to select more than one.
Groups:
Available groups
Filter
Reviewer
Operator
Scientist
Chosen groups
Choose all
Remove all
Specific permissions for this user. Hold down "Control", or "Command" on a Mac, to select more than one.
User permissions:
Available user permissions
Filter
auth | user | Deny analysis editing
auth | user | Deny copy, export of data
auth | user | Deny instrument administrati
auth | user | Deny instrument control
auth | user | Deny plate editing
auth | user | Deny protocol editing
auth | user | Deny sign off of data
auth | user | Allow analysis editing
auth | user | Allow copy, export of data
auth | user | Allow instrument administrati
auth | user | Allow instrument control
auth | user | Allow plate editing
auth | user | Allow protocol editing
Chosen user permissions
Choose all
Remove all
Important dates
Last login:
Date: 2013-06-11 Today
Time: 08:19:34 Now
Date joined:
Date: 2013-06-11 Today
Time: 08:19:34 Now
Delete
Save and add another
Save and continue editing
Save

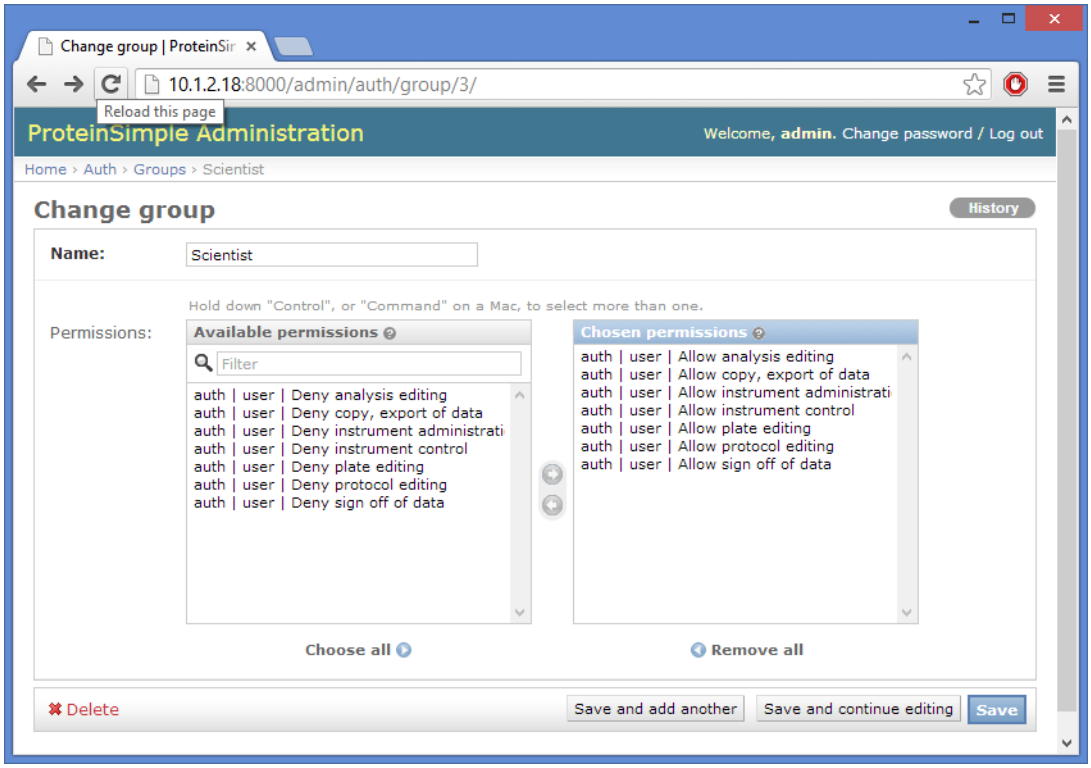
Use the Groups page to change the permissions in a group or create new groups:



To change permissions for a group click **Change**, then select a group:



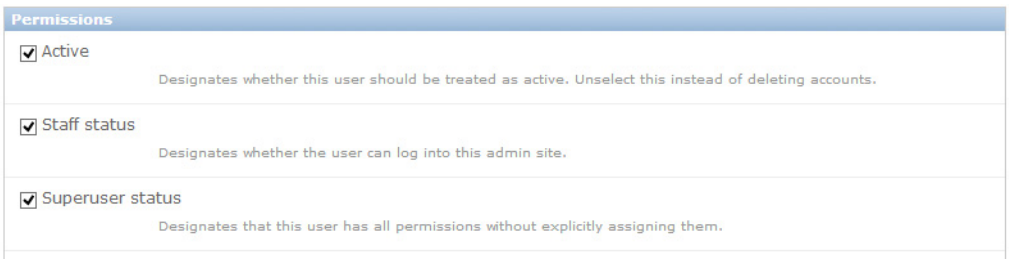
Move individual group permissions in or out of the Available Permissions and Chosen Permissions boxes by selecting a permission in either box. Click the **left** or **right** arrow button to move the permission into the other box.


Change group | ProteinSir
10.1.2.18:8000/admin/auth/group/3/
Reload this page
ProteinSimple Administration
Welcome, admin. Change password / Log out
Home › Auth › Groups › Scientist
Change group
History
Name:
Scientist
Hold down "Control", or "Command" on a Mac, to select more than one.
Permissions:
Available permissions
Filter
auth | user | Deny analysis editing
auth | user | Deny copy, export of data
auth | user | Deny instrument administrati
auth | user | Deny instrument control
auth | user | Deny plate editing
auth | user | Deny protocol editing
auth | user | Deny sign off of data
Chosen permissions
auth | user | Allow analysis editing
auth | user | Allow copy, export of data
auth | user | Allow instrument administrati
auth | user | Allow instrument control
auth | user | Allow plate editing
auth | user | Allow protocol editing
auth | user | Allow sign off of data
Choose all
Remove all
Delete
Save and add another
Save and continue editing
Save

Adding Admin Users

To create a user with administrator permissions:

1. Follow the steps described in "Adding Non-admin Users" on page 412 to create the admin user.
2. Under permissions, select **Staff status** and **Superuser status**:


Permissions
Active
Designates whether this user should be treated as active. Unselect this instead of deleting accounts.
Staff status
Designates whether the user can log into this admin site.
Superuser status
Designates that this user has all permissions without explicitly assigning them.

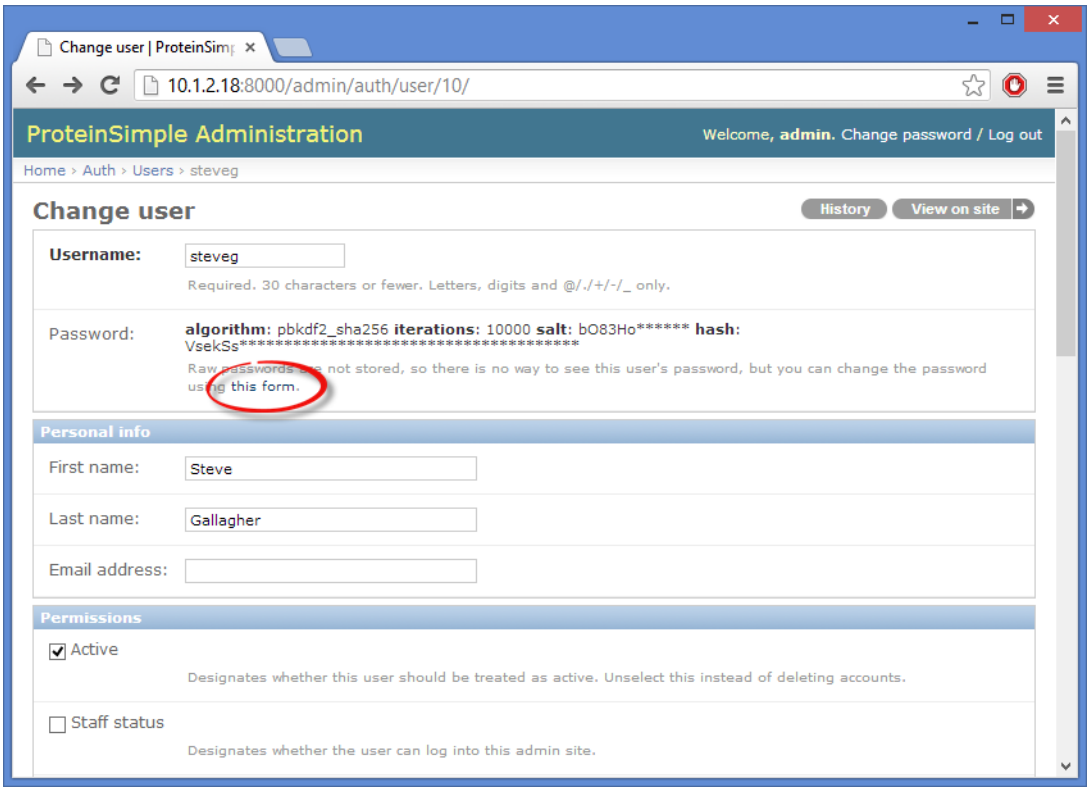
3. Assign the admin user to a group.

NOTE: Selecting Superuser status enables server permissions only. Admin users must be also be assigned to a group to in order to have Compass permissions.

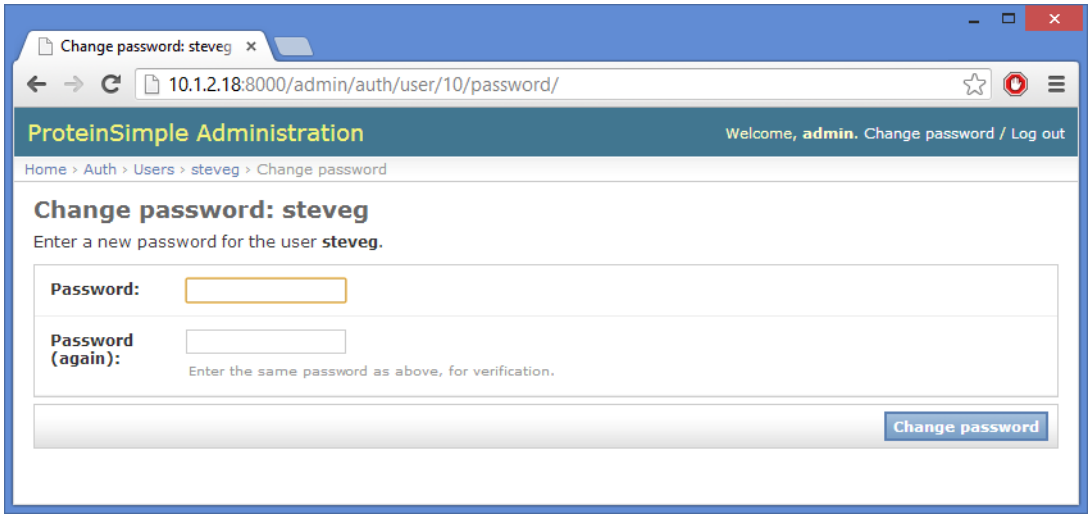
Resetting User Passwords

To reset a user password:

1. Select **Users** from the Site Administration home page, then select the user to change. The following screen displays:


Change user | ProteinSim
10.1.2.18:8000/admin/auth/user/10/
ProteinSimple Administration
Welcome, admin. Change password / Log out
Home › Auth › Users › steveg
Change user
History
View on site
Username:
steveg
Required. 30 characters or fewer. Letters, digits and @/./+/-/_ only.
Password:
algorithm: pbkdf2_sha256 iterations: 10000 salt: bO83Ho****** hash:
VsekSs**********
Raw passwords are not stored, so there is no way to see this user's password, but you can change the password using this form.
Personal info
First name:
Steve
Last name:
Gallagher
Email address:
Permissions
Active
Designates whether this user should be treated as active. Unselect this instead of deleting accounts.
Staff status
Designates whether the user can log into this admin site.

2. Raw passwords are not stored, they must be changed manually. Click the text link to access the password change form:


Change password: steveg
10.1.2.18:8000/admin/auth/user/10/password/
ProteinSimple Administration
Welcome, admin. Change password / Log out
Home › Auth › Users › steveg › Change password
Change password: steveg
Enter a new password for the user steveg.
Password:
Password (again):
Enter the same password as above, for verification.
Change password

3. Enter the new password, then click **Change password**.

Encryption Details

Compass uses the SHA1 hash algorithm to generate a 160 bit hash code that is unique for all files. All files saved by Compass are encrypted with a digital key. This key along with the hash codes guarantees the file history is correct and no other edits were made. All changes saved to a file have the electronic signature of the user who saved the file. The **e-Signature** command allows a user to sign off on a state such as approved or verified.

There is no individual ownership of files, all users who log into Compass can open any file.